PARASITOLOGY

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PARASITOLOGY

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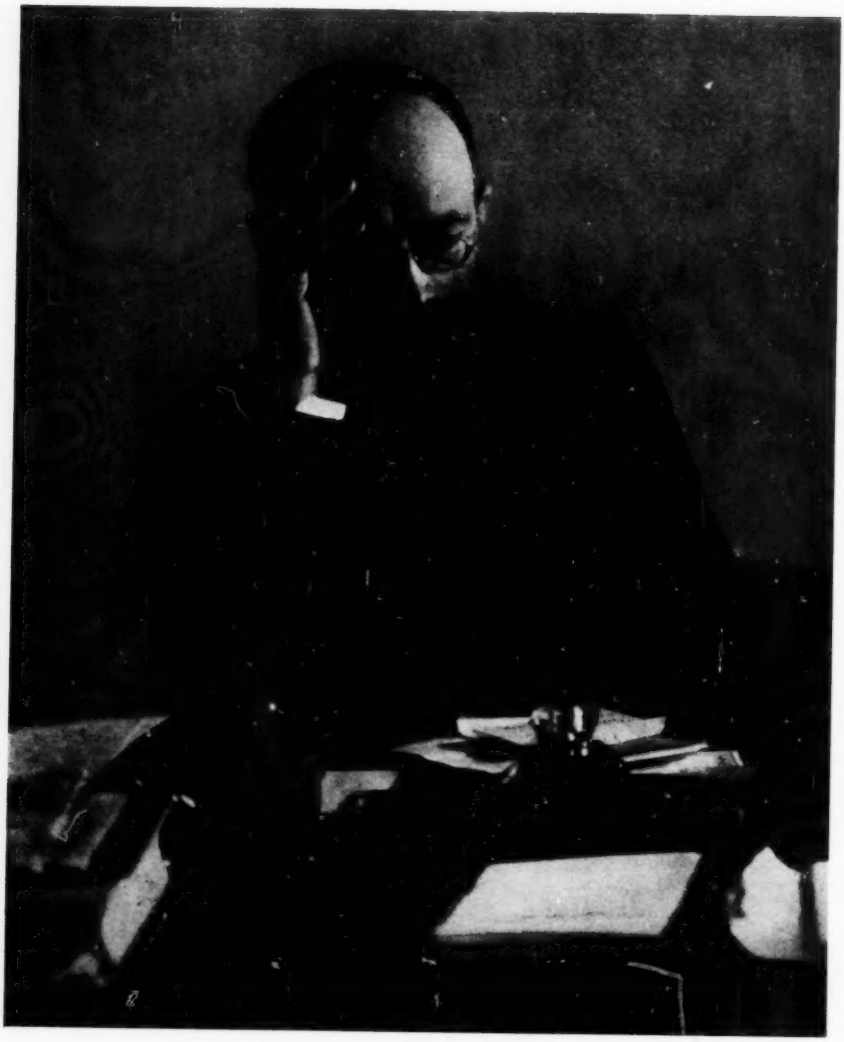

Prof. dr Michał Siedlecki
5/V 1920. Cracow - Poland.

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MICHAŁ SIEDLECKI (1873–1940)

A FOUNDER OF MODERN KNOWLEDGE OF THE SPOROZOA

BY CLIFFORD DOBELL, F.R.S.
National Institute for Medical Research, London, N.W. 3

(With Plate I)

ON 6 November 1939, shortly after the invasion of Poland, the professors at the Jagellonian University of Cracow were summoned to attend a lecture on "Nazi Science", to be delivered by a high German official. Those who obeyed the order were arrested. The charge brought against them was, in brief, that they had been attempting to fulfil their duties at the University, and were thereby guilty of striving to keep alight the flame of Polish national culture. For these offences the unfortunate professors—some of them old and feeble—were imprisoned and robbed of their property. After being jailed in Cracow they were taken to a convict prison at Breslau and thence to a concentration camp at Sachsenhausen-Oranienburg (near Berlin)—where, of course, many of them died.

Among the unfortunates who perished at Sachsenhausen was Prof. M. Siedlecki, the distinguished zoologist. The circumstances and even the date of his death are not at present known with certainty outside Germany—indeed, they may never be known; but it is believed that he died of heart-failure—due to exposure during the past bitter winter—in January 1940.[1] As Siedlecki was one of the founders of modern knowledge of the Sporozoa, I think readers of *Parasitology*—in all civilized countries—will welcome the accompanying reproduction of his portrait[2] (Pl. I) and the following notes on his career and his contributions to Protozoology. I publish them as a tribute to the memory of a great original investigator, whose early writings have been an inspiration throughout my own working life.

Michał Siedlecki[3] was born, of good Polish ancestry, at Cracow [Kraków, then in Austria] in 1873, and passed most of his life in that famous city. He was a student at its ancient University, and there took his doctor's degree in 1895. In the following year (1896) he studied at the Zoological Institute in

[1] The first intimation I had of his death was from the *Daily Telegraph* (31. i. 40), whose Copenhagen correspondent briefly reported that "Prof. Michael Siedlecki...has, I learn, died of ill-treatment in the Nazi concentration camp at Sachsenhausen".

[2] The original may be seen in the Molteno Institute at Cambridge. I also possess a copy which Siedlecki gave me in 1926. Another portrait, of much later date, has recently appeared in the *Journal of the Society for the Preservation of the Fauna of the Empire*, Part xxxix (N.S.), April 1940 (opp. p. 16).

[3] His real forename was that given above; but he was accustomed to translate it (Michel in French, Michael in English, etc.) when writing in foreign languages.

Berlin (where F. E. Schulze was Professor), and then spent the remaining years of the century working under Metchnikoff at the Pasteur Institute in Paris and at the Zoological Station in Naples (then run by Anton Dohrn, of blessed memory). It was during this brief period (1896–9) that Siedlecki carried out the researches on the Sporozoa which have made his name famous to all protozoologists. After these fruitful excursions abroad he returned to Cracow and remained there, with few interruptions, for the rest of his life. In 1900 he was appointed lecturer at the University, and in 1912 he duly succeeded A. Wierzejski as Professor of Zoology and Director of the Zoological Laboratory and Museum. This position he held to the end of his days—with an interval (1919–21) as Rector of the University of Vilna [Wilno] during its reconstruction after the last war.

Siedlecki's zoological interests were not confined to the Protozoa. His first published paper (1895) dealt with the leucocytes of Urodela, and he afterwards studied the phagocytes of Annelids (1903) and Echinoderms (with Caullery, 1903). With Kostanecki he early published an important work on the cytology of *Ascaris* (1896), while later he devoted much attention to the biology and reproduction of the tropical flying-frog (*Rhacophorus*), which he studied during an expedition to Java (1908–9). Two once well-known papers with Krzyształowicz (1905, 1908) on the spirochaete of syphilis [*Treponema pallidum*] may also be noted here—the parasite being then wrongly regarded as a flagellate. During the latter half of his life he became interested in marine biology (especially fishes) and ornithology, and for many years was Polish representative on the *Conseil Permanent International pour l'Exploration de la Mer* and the International Committee for Bird Preservation. He played an important part in the development of Polish fisheries in the Baltic and the North Sea, and was instrumental in establishing marine biological stations at Hel and Gdynia (now destroyed). The conservation of wild life in his own country, and the scientific exploitation of its natural resources, were matters dear to his heart.

Siedlecki's pioneer work on the Sporozoa (Coccidia and Gregarines) was all done when he was a young man, and his fundamental discoveries were partly made in collaboration with another and more famous protozoologist. To appreciate his own share it is thus necessary to know the details, which I may now briefly recall.

When Siedlecki went to Berlin in 1896 he intended to study the Foraminifera under Fritz Schaudinn (Assistant to Prof. Schulze), who had then just announced some remarkable discoveries relating to these organisms. But at Schaudinn's suggestion, and in order to learn his methods, he embarked instead upon a joint inquiry into a very different subject—the life-histories of the coccidia living in centipedes (*Lithobius forficatus*). At this date Siedlecki was only 23, and his mentor but 2 years older:[1] yet within a few months these

[1] Schaudinn was born 19 September 1871 and died 22 June 1906. He published his first paper in 1893, and took his doctor's degree in 1894—only a year before Siedlecki.

two young men succeeded in solving the riddles of the coccidian life-cycle so effectively that everything which has since been found out is merely an elaboration of detail. Before 1896 all the main facts were, indeed, known; but they could not be pieced together properly. Schaudinn and Siedlecki, for the first time, identified or discovered each isolated bit of the jig-saw puzzle, and combined them all into a complete and convincing picture. They thus produced order out of chaos, and laid a solid foundation for all future work. Their joint preliminary paper was read at a meeting of the German Zoological Society in June 1897, and is now one of the classics of Protozoology.

But Schaudinn and Siedlecki never published a full account of their work together. At the beginning of 1897 Siedlecki left Berlin and went to Naples, while soon afterwards Schaudinn—who was also busy with other important investigations—had to do his military service; so the two friends decided to publish their final results separately. As they had found coccidia of two different genera (*Adelea* and *Coccidium* [=*Eimeria*]) in their centipedes, each worker undertook to describe one—Schaudinn taking *Coccidium* and Siedlecki *Adelea*. At Naples, however, Siedlecki was able to confirm their findings, in part, by a study of another form living in cuttle-fish—*Aggregata* [then known as *Klossia* or *Benedenia*]. His accounts of this parasite were published in 1898, and that of *Adelea* was delayed until the following year. The material for all these papers (Siedlecki, 1898, 1898*b*, 1899) was worked up at the Pasteur Institute in Paris, with the assistance of Félix Mesnil. Schaudinn's celebrated description of the life-history of *Coccidium* did not appear until 1900.

All the papers just referred to are now protozoological classics. It is often stated that Schaudinn's final monograph (1900) contains the first complete account of the life-history of any coccidian, but—as will be evident—this is incorrect. It is correct to say that the life-cycle of the Coccidia, in general, was finally elucidated by Schaudinn and Siedlecki jointly (1897), while the latter actually published the first complete account of any species (*Adelea ovata*, 1899). Siedlecki's description of "*Klossia*" [=*Aggregata*]—the first complete account of the sexual cycle—even preceded this by several months. And moreover this was all his own work—a beautiful piece of research which has since been amply confirmed in every essential.

The Coccidia living in *Lithobius forficatus* were not completely described by Schaudinn and Siedlecki, and later work has shown that the problem is even more complex than they imagined. In their preliminary paper (1897) they described only 2 species—identified by them as *Coccidium* (or *Eimeria*) *schneideri* and *Adelea ovata*. Before he published his final paper on *Coccidium*, however, Schaudinn discovered that there were really 2 species of this genus in their centipedes—"*C. schneideri*", which he reidentified as *C. lacazei* [now known as *Eimeria lacazei*], and a new species which he named *C. schubergi* [later generally known as *Eimeria schubergi*, and now renamed *E. schaudinniana* (Pinto, 1928)]. It is with this species—not studied by Siedlecki—that Schaudinn's masterpiece (1900) chiefly deals. Moreover, it has since been shown by Schellack & Reichenow (1913, 1915) that in *Lithobius* there is even a fourth species belonging to yet another genus (*Barrouxia*), which Schaudinn and Siedlecki unaccountably overlooked, though some stages of its schizogony were wrongly incorporated in their accounts of *Adelea*.

As regards the coccidian of the cuttle-fish (*Aggregata eberthi*), it is now known that Siedlecki's account covered only half its life-history. He gave a complete description of the sexual cycle in *Sepia*—which he believed to be the whole life-history—but it has since been shown by Léger & Duboscq (1908) that the parasite undergoes also an asexual development in crabs (*Portunus*). This work has been fully confirmed (cf. Dobell, 1925).

The chief addition to knowledge of coccidian life-history, since Siedlecki's day, has been the demonstration that the Coccidia are haploid organisms, with constant chromosome numbers and zygotic reduction[1]—a fact unsuspected when Schaudinn and Siedlecki wrote.

In addition to his works on the coccidia of centipedes and cuttle-fish, Siedlecki wrote valuable papers on other species.[2] In 1898 he described (see Siedlecki, 1898*a*), for the first time, the fertilization of *Coccidium proprium* [now called *Eimeria propria*] from newts [*Triton*=*Molge*]; while later (1902, 1907) he gave a complete account of the life-cycle of *Caryotropha mesnili*—a remarkable form which he discovered in the male germ-cells of a marine polychaete (*Polymnia*). The material for this research was collected—in the post-Schaudinnian period—at Naples, Wimereux, and Trieste. It may be added, in passing, that in 1902 Siedlecki also described a curious astomatous ciliate (*Herpetophrya*) which he found in the same host.

During his stay at Naples from November 1898 till July 1899, Siedlecki obtained the material for his only publication on the Gregarines—his paper on *Monocystis ascidiae* [now known as *Lankesteria ascidiae*], a common parasite of *Ciona*. He completed this work in Prof. Hoyer's laboratory at Cracow, and published it at the end of 1899. The paper (Siedlecki, 1899*a*) deals with the sexual cycle of *Lankesteria*, and Siedlecki believed that he had studied only a part of its life-history; but in fact he described the whole, and described it exactly. Jameson (1920), writing 20 years later, said truly of this "most excellent account" that it "outlined the course of gregarine development in a masterly fashion and left only the details to be filled in". The most important detail was filled in by Jameson himself,[3] who showed that the Gregarines—or many of them, at least—are, like the Coccidia, haploid organisms with post-meiotic reduction.

Just as Schaudinn and Siedlecki together produced order out of chaos in our knowledge of the Coccidia, so Siedlecki alone clarified, at a stroke, the muddled conceptions then prevailing about the development of the Gregarines. When two people publish a piece of work together, there must always be some doubt regarding the share of credit due to either; and consequently we cannot now say whether Schaudinn or Siedlecki first elucidated the life-history of the Coccidia. Apparently they did it jointly, so our gratitude should be expressed to both equally. Unfortunately this is seldom done, and Siedlecki's solid achievements have thus been overshadowed by the fame of his East-Prussian collaborator. But as regards the Gregarines there is and can be no un-

[1] See Dobell & Jameson (1915), Dobell (1925).

[2] A genus *Siedleckia* was introduced for a very remarkable sporozoon by Caullery & Mesnil in 1898: but this was not a form which he himself studied.

[3] See Dobell & Jameson (1915), Jameson (1920).

certainty. Schaudinn never wrote anything original about these organisms:[1] Siedlecki—alone and unaided—laid the foundations of modern knowledge in 1899.

For two generations elementary students of Zoology have been taught the life-histories of "Coccidium" and "Gregarina" as outlined originally by Schaudinn and Siedlecki, but they seldom realize—any more than their teachers—how tremendous a transformation these two young men wrought in our knowledge of the Sporozoa. Yet one has only to compare Wasielewski's *Sporozoenkunde* (1896) with Minchin's "Sporozoa" in Lankester's *Treatise on Zoology* (1903) to see the results of the revolution. The first work, though good for its period, is inchoate, disjointed, and hard to comprehend: the second presents the subject in beautiful order—with everything definite and in its proper place, so that everyone who reads may understand. The difference is not due solely to Minchin's greater powers of exposition: it is also due largely to Schaudinn's and Siedlecki's intervening discoveries, and Schaudinn's exceptional ability to exploit them. Of Siedlecki's contribution it may be justly said that his work has now so completely permeated the *corpus* of modern protozoological knowledge that its origin is almost forgotten and its correctness is no longer questioned. This is surely the hall-mark of fundamental scientific research.

The observations of Schaudinn and Siedlecki on coccidia and gregarines have an interest which is not merely academic; for they illuminated and explained the simultaneous discoveries of Ross and Grassi regarding the parasites of malaria, and thus played an all-important part in establishing our present knowledge of these organisms. Without the fundamental researches of Siedlecki it would have been impossible, forty years ago, to understand the complicated life-cycle of *Plasmodium*. For this application of his work alone, therefore, Siedlecki has earned the gratitude of mankind. Yet it is possible that he hardly realized the full significance of his own discoveries, and the magnitude of their implications; for he was a simple and modest man, who—unlike some of his contemporaries—never boasted of his prowess or advertised his achievements.

I never had the luck to meet Siedlecki in the flesh; consequently, all I know about him personally is derived from the study of his publications and desultory correspondence during the last 30 years—supplemented by the reports of mutual friends. Everybody who knew him remarked his scientific and administrative ability, his good breeding, his fervid patriotism. In an anonymous obituary (*Nature*, **145**, 963: 22 June 1940) it is recorded that "he was deeply respected by everyone with whom he came in contact, of whatever nationality,[2] and those who had the privilege of working with him

[1] It may be of interest to add that Schaudinn began to study the gregarines of *Lithobius*, but never finished these researches. It was in the course of this study that he encountered the coccidia which inspired his work with Siedlecki.

[2] Except one? Perhaps the exception proves the rule.

will always remember him with a lasting affection." Miss Phyllis Barclay-Smith adds (*in litt.*): "His modesty, his great width of vision and understanding, and his gaiety were exceptional. He was interested in everything, and I think everyone...at once felt his charm." D'Arcy Thompson—who saw much of him on the *Conseil International*—writes to me: "He was a little, quiet, intensely cheerful and happy man...without an enemy[1] or a hard thought of anyone. He was a sort of polymath, knowing all sorts of unexpected things. He spoke English admirably, German (of course) perfectly: and he could make shift to speak I don't know how many tongues besides." From my own experience I can attest that he was scientifically modest about his accomplishments—always ready to accept competent criticism and correction, never claiming priority or prestige, and benevolent to younger and less gifted workers. A few lines from a letter which he wrote to me on 7 January 1926—*à propos* of my confirmations and criticisms of his work on *Aggregata*—will serve in illustration of this aspect of his personality. He wrote (in English): "I am aware that every scientific work, and especially the biological ones, can be repeated after some years and always new facts can be discovered and new ideas introduced. Science is in continuous progress, the manner of thinking changes, the methods are developed; and therefore I consider it as quite natural that new works contain critical remarks concerning the older ones. But, really, it is a great satisfaction to see that, after a careful study made by [a]...specialist, the main framework of my study remains nearly untouched ...I accept justified criticisms always gratefully, because I am conscious that they indicate a new progress of Science."

The main framework of Siedlecki's study of the Sporozoa still remains, indeed, almost untouched. His poor old Polish body has been wantonly destroyed, but his works and his spirit are inviolable and unconquerable and will march proudly on for ever in the service of science and humanity.

For some of the biographical information in the foregoing article I am indebted to friends and correspondents—especially Prof. Count K. Wodzicki, Sir D'Arcy Thompson, F.R.S., and Miss Phyllis Barclay-Smith (Secretary of the International Committee for Bird Preservation). To these I offer my thanks once more. My estimate of Siedlecki's work is based upon personal study of his publications and the organisms which he investigated, and the literature on the Sporozoa from its beginnings to the present day.

[1] Cf. preceding footnote, and the first paragraph of this article.

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(*MS. received for publication* 9. VIII. 1940.—Ed.)

THE BIOLOGY AND POST-EMBRYONIC DEVELOPMENT OF *OPIUS ILICIS* N.SP., A PARASITE OF THE HOLLY LEAF-MINER (*PHYTOMYZA ILICIS* CURT.)

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(With Plate II and 8 Figures in the Text)

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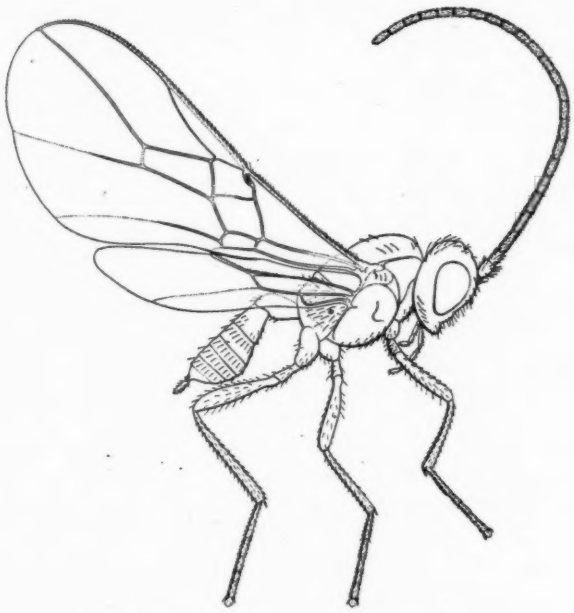
I. Introduction

In the course of the writer's work on the biological control of the holly leaf-miner (*Phytomyza ilicis* Curt.), an insect which has been causing considerable damage to holly in western Canada and other parts of the world, a new species of *Opius*, was reared from several of the fly puparia. Although this Braconid is not a very common parasite of *Phytomyza ilicis* and on this account was only briefly mentioned in the author's previous paper (1939) dealing with the numerous Chalcid parasites of this host, yet it is of sufficient general interest, apart from the fact of its being new to science, to merit separate descriptive treatment. The present paper, therefore, gives a more or less complete account of its systematics, biology, morphology—more especially of the post-embryonic stages—anatomy, distribution, and host relationship. Its value as a parasite of the holly leaf-miner, the subject of interspecific competition, and some interesting phases of larval development which are of general entomological importance, are also discussed.

II. SYSTEMATIC NOTES

Opius ilicis (Text-fig. 1) belongs to the family Braconidae, division Polymorphi, and tribe Opiinae. The Polymorphi, according to Marshall (1891), are characterized by the rigid suturiform articulation between the second and third abdominal segments, the unemarginate clypeus, and the cubital areolet, which is large, quadrangular, or wanting, but not minute, as in the Areolarii, while the Opiinae can be distinguished by the concave occiput, the three cubital areolets in the forewing, the axillary areolet which is without



Text-fig. 1. *Opius ilicis*, adult female (× 23).

a vestige of a transverse nervure, and the subsessile and ovate abdomen. *Opius* itself differs from the six remaining Opiine genera in the following particulars: the closed radial areolet, the radius, which springs from the base of the stigma; the second radial abscissa which is longer than the first intercubital nervure; and the narrow elongate stigma.

Mr G. Nixon, the Braconid expert of the Imperial Institute of Entomology, who examined the material I had reared, agreed at my suggestion to give this new parasite the specific name of *ilicis* Nixon, thus indicating its relationship with the host *Phytomyza ilicis*. The following short description of the imago, which was prepared by Mr Nixon and published recently in the

Entomologist's Monthly Magazine (1939), is reproduced here in order to complete the general account of the species:

"♂, ♀. Head brownish black above; the orbits, face, temples and cheeks pale brownish yellow; sometimes the face is suffused with darker colouring. Thorax brownish black; mesopleurae below sometimes suffused with reddish, as is also the mesosternum. Legs unicolourous, pale yellow. In examples caught wild the yellow colouring is more intense, almost ochreous, and the black patch on the top of the head tends to be reduced in size. On the whole there seems to be considerable variation in the colour of the head and the abdomen.

"♀. *Head:* Apex of clypeus widely separated from the mandibles. Mandibles with their lower margin simple; no trace whatever of an angulation near the base. Antennae with 23–29 segments (23–26 in 5 bred ♀♀; 26–29 in 9 wild ♀♀). *Thorax:* Notauli virtually wanting, showing anteriorly as short, deep, more or less smooth niches; the anterior margin of these niches is raised so that the mesonotum has prominent 'shoulders'. In bred ♀♀ the mesonotum is feebly longitudinally impressed; this feature is much less in evidence in wild examples. A few long hairs are present along the imaginary course of the notauli, especially posteriorly. No trace of a fovea against the posterior margin of the mesonotum. Posterior margin of the scutellum margined by a long, narrow, finely crenate groove. Mesopleurae with only a feeble, completely smooth impression. Propodeum predominantly smooth and shining. *Forewings:* radius leaving the stigma far proximal to middle; nervus parallelus arising from discoidalis very near middle of outer side of second discoidal cell. *Abdomen:* Petiole about one-and-a-half times as long as its apical width, finely rugose outside the area enclosed by the basal carinae and with a longitudinal element in the sculpture. Each of the tergites with a fairly even row of long cilia. Ovipositor projecting slightly beyond the apex of the abdomen.

"♂. Antennae with 25–28 segments (18 ♂♂).

"Length. ♂, ♀, about 1·8 mm.

"This species is superficially very like *Opius compar* Marshall, the type of which is in the British Museum. The most obvious differences between the two species are as follows: *O. compar* has the head thicker, less transverse, a small fovea against the posterior margin of the mesonotum, and the nervus parallelus arising from nearer the lower exterior angle of the second discoidal cell. I have not examined the mandibles of *O. compar* Marsh., since the only available specimen, the type female, is mounted flat on a card. Colour, that is the contrast between yellow and dark markings, is a much more striking and characteristic feature of *O. ilicis* n.sp. than of *O. compar* Marsh., and is a very valuable guide to the identification of the species."

III. DISTRIBUTION AND HOST RECORDS

Specimens of *O. ilicis* were reared by the writer from holly fly material collected at Farnham Royal, Bucks; Windlesham, Surrey; Sunninghill, Berks; and the New Forest in Hampshire. This parasite was most abundant at Windlesham, Surrey, in an area where holly was associated with Scots pine. The parasitism in this area in 1939 averaged 4%, that is to say four *Opius* adults emerged from one hundred mines. By dissection in the early spring, however, 7% of the host larvae were found to be parasitized, but this figure, as a result of the pressure exerted by the intrinsically superior Chalcid, *Chrysocharis gemma*, was ultimately reduced to 4%. At Farnham Royal not more than 1% of the mines were attacked, and in several other areas, including Burnham Beeches in Buckinghamshire, *Opius ilicis* was not represented in the sample collections. From the very large consignments of holly fly material shipped to Canada from the latter region in 1939, however, a few specimens of this parasite were obtained (11 out of 100,000 mines), and this would seem to indicate that although *O. ilicis* is very rare in certain holly areas, it is, nevertheless, not completely absent from them.

The distribution of the genus *Opius* is world-wide, and numerous Opiine species have been reared from a large number of hosts in all five continents. In temperate regions the insects which suffer most from their attacks are species of *Pegomyia*, *Agromyza*, *Rhagoletis*, *Phytomyza* and *Cerodonta*, whilst in tropical and subtropical areas the most favoured hosts belong to one or other of the two genera *Dacus* and *Anastrepha*. It should be noted that all these insects are members of the order Diptera. Further host records are included in the succeeding section.

IV. HOST RELATIONSHIP OF THE GENUS *OPIUS*

"The Opiinae", states the Rev. T. A. Marshall in his admirable *Monograph of British Braconidae* (1891), "is one of the most neglected of all Hymenopterous tribes." Why this should be so is not quite clear, for this group contains an interesting, and, from the economic point of view, a very important collection of parasites. It is therefore to be hoped that the following data on the host relationship of the genus *Opius*, which has been collected for, among other reasons, the assistance of workers on Opiine species, will help to direct the attention of taxonomists towards them, and thus lead to a better arrangement of the whole group.

Specialists in the Braconidae are generally agreed that the genus *Opius*, as it stands at present, contains a large number of incorrectly determined species, some of which almost certainly belong to quite different genera, while several more have probably been given wrong generic names, so that a lot of taxonomic work still remains to be done before the various members of this large genus can be considered to have been satisfactorily classified. It is the

opinion of the present writer that a good general knowledge of the host relationship of the known species of a genus, that is, in so far as the parasites have been reared from their hosts and the relationship recorded in the literature, will be of great value to the systematist, especially when he is faced with the determination of doubtful and aberrant species. After all, the ideal scheme of classification should, if at all possible, take into account not only morphological, but also physiological and particularly ecological data, and it is with this conception in mind that the following list of host records for the genus *Opius* has been compiled. But apart altogether from classification the subject of host relationship is extremely interesting, especially from the economic standpoint, and any increase in our knowledge of the type of hosts attacked by a particular group of parasites is bound to be of sound practical value to the worker in biological control. This is especially true of the genus under review because of the very large number of economic pests which its members attack.

In the following list which has been compiled from various sources, including the Farnham House Catalogue, Leonardi, Essig, etc., there are 205 host records. The number of identified species involved is ninety-one, while twenty-one more are simply recorded as *Opius* sp. In the host list are included representatives of the five chief orders of the Insecta—Diptera, Lepidoptera, Coleoptera, Hemiptera, and Hymenoptera. By far the greatest number belong to the order Diptera, their relative abundance being as follows:

(1) Diptera with 182 records.
(2) Lepidoptera with twelve records.
(3) Coleoptera with nine records.
(4) Hemiptera with one record.
(5) Hymenoptera with one record.

The genera which suffer most heavily from the attacks of these parasites in the order of their frequency are as follows: (1) *Pegomyia* (36 records); (2) *Agromyza* (29 records); (3) *Dacus* (25 records); (4) *Ceratitis* (16 records); (5) *Rhagoletis* (10 records); (6) *Phytomyza* (9 records); (7) *Anastrepha* (8 records); (8) *Cerodonta* (4 records); while the most heavily attacked species are the Anthomyiid, *Pegomyia hyoscyami*, whose larvae mine the leaves of beet and mangold, and the Trypetid, *Dacus oleae*, the immature stages of which feed on the fruit of the olive tree. From the former, fourteen distinct species of *Opius* have been reared, in countries as widely separated as England, Germany, Sweden, Holland, Belgium, Russia, Italy, Canada, and the United States of America, while from the latter six species are recorded, mostly from the Mediterranean littoral, South Africa and India. It is particularly interesting to notice that the larvae which are attacked by the various species of *Opius* can usually (but not always) be found in one of two particular types of habitat. For the most part they are either leaf-miners or feeders inside fruits.

In the following list, all hosts not otherwise indicated, belong to the order Diptera:

Opius afreutretae Wlkn.
South Africa: *Acanthiphilus muiri* Bezzi, *Afreutretae bipunctata*, and *A. discoidalis* Bezzi.
Opius africanus Sz.
Italy, Eritrea, and South Africa: *Dacus oleae* Gmel.
Opius africanus var. *orientalis* Silv.
Eritrea and South Africa: *Dacus oleae* Gmel.
Opius agromyzae Vier.
Italy: *Agromyza nigripes* Mg. North America: *A. pusilla* Mg.
Opius ambivius Gour.
France and Italy: *Phytomyza ancholiae* R.-D. France: *P. xylostei* R.-D.
Opius anastrephae Vier.
Porto Rico: *Anastrepha* sp. Jamaica and Porto Rico: *A. fraterculus* Wied. U.S.A.: Trypetid species.
Opius anthomyiae Ashm.
U.S.A.: Anthomyiid species. North America: *Pegomyia bicolor* Wied. and *P. hyoscyami* Panz.
Opius aridis Gahan
North America: *Agromyza pusilla* Mg. U.S.A.: *A. scutellata* Fall., and *Cerodonta dorsalis* Lw.
Opius arisanus Sonan
Formosa: *Chaetodacus ferrugineus* var. *dorsalis* Hendel, and *Dacus dorsalis* Hend.
Opius bellus Gahan
Panama Canal Zone: *Anastrepha fraterculus* Wied.
Opius betae Bengtsson
Sweden: *Pegomyia hyoscyami* Panz.
Opius bremeri Bengtsson
Germany: *Pegomyia hyoscyami* Panz.
Opius bruneipes Gahan
North America: *Agromyza pusilla* Mg.
Opius brunneus Gour.
Italy: *Coleophora serenella* Z. (Lepidoptera).
Opius carbonarius Nees
Germany and Sweden: *Pegomyia hyoscyami* Panz.
Opius carinatus Thoms.
Germany: *Plodia interpunctella* Hb. (Lepidoptera).
Opius carpomyiae Silv.
India: *Carpomyia vesuviana* Costa and Trypetid species.
Opius caudatus Wesm.
France: *Callidium* sp. (Coleoptera) and *Pogonochaerus* sp. (Coleoptera). Italy: *Pogonochaerus hispidis* L. (Coleoptera), and *Pyrrhidium sanguineum* L. (Coleoptera).
Opius cereus Gahan
Trinidad: *Anastrepha serpentina* Wied. and *A. striata* Schin.
Opius cingulatus Wesm.
Europe: *Agromyza morio* Bris. Britain: *Acidia heraclei* L.
Opius compensans Silv.
India: *Dacus incisus* Wlk.
Opius concolor Sz.
Eritrea: *Carpomyia incompleta* Beck. Palestine, Tripoli, Algeria, Morocco, Italy, France, Greece, North Africa, and Tunisia: *Dacus oleae* Gmel.

Opius coriaceus Gahan
North America: *Cerodonta femoralis* Mg. U.S.A.: *C. dorsalis* Lw.

Opius cosyrae Wlkn.
Tanganyika: *Ceratitis cosyra* Wlk.

Opius cupidus Gahan
U.S.A.: *Pegomyia hyoscyami* Panz.

Opius dacicida Silv.
Italy, Eritrea and Transvaal: *Dacus oleae* Gmel.

Opius diastatae Ashm.
North America: *Agromyza parvicornis* Lw.

Opius dimidiatus Ashm.
U.S.A.: *Agromyza* sp., *A. pusilla* Mg., *A. scutellata* Fall. and *Cerodonta dorsalis* Lw.

Opius downesi Gahan
North America: *Rhagoletis pomonella* Walsh.

Opius ferrugineus Gahan
Canada: *Rhagoletis cingulata* Lw. and *R. fausta* O.S. U.S.A.: *R. pomonella* Walsh.

Opius fijiensis Fullaway
Fiji: *Dacus* sp., *D. passiflorae* Frogg., and Trypetid species.

Opius fletcheri Silv.
India: *Bactrocera cucurbitae* Coq. and *Carpomyia vesuviana* Costa. Hawaii, India, Loochoo Is., and Malaya: *Dacus cucurbitae* Coq. Malaya: *D. ferrugineus* F.

Opius foersteri Gahan
U.S.A.: *Eulia velutinana* Wlk. (Lepidoptera).

Opius formosanus Fullaway
Formosa: *Chaetodacus ferrugineus* F., *C. ferrugineus* var. *dorsalis* Hendel, and *Zeugodacus synnephes* Hendel.

Opius foveolatus Ashm.
North America: *Pegomyia hyoscyami* Panz.

Opius fulvicollis Thoms.
Belgium: *Pegomyia hyoscyami* var. *Betae* Curt. Sweden, Germany, U.S.A., North America, Holland and Belgium: *P. hyoscyami* Panz.

Opius geniculatus Thn.
Germany: *Stemnocera abrotani* Mg.

Opius giffardi Silv.
Tanganyika: *Ceratitis capitata* Wied.

Opius graccus Wesm.
Italy: *Nematus quercus* Htg. (Hymenoptera).

Opius humilis Silv.
Hawaii, Bermuda, Tunis, Spain, Kenya, Africa, and Hawaii to Australia: *Ceratitis capitata* Wied. Kenya: *C. cosyra* Wlk. Hawaii: *Dacus cucurbitae* Coq. U.S.A.: *Rhagoletis suavis completa* Cress.

Opius hyoscyamiellus Vier.
Canada: *Pegomyia hyoscyami* Panz.

Opius incisi Silv.
India: *Dacus incisus* Wlk.

Opius insularis Ashm.
Porto Rico: *Agromyza* sp.

Opius irregularis Wesm.
France: *Pegomyia acetosae*. Italy: *P. abbreviata* Pck.

Opius lantanae Bridw.
Hawaii: *Oscinis* sp.

Opius lectoides Gahan
U.S.A.: *Rhagoletis pomonella* Walsh.

Opius lectus Gahan
U.S.A.: *Rhagoletis pomonella* Walsh.

Opius leptostigma Wesm.
Italy: *Phora tuberum* Gour.

Opius longistigmus Gour.
France: *Phora tuberum* Gour.

Opius makii Sonan
Formosa: *Chaetodacus ferrugineus* var. *dorsalis* Hendel and *Dacus dorsalis* Hendel.

Opius mandibularis Gahan
North America: *Phytomyza chrysanthemi* Kowarz. U.S.A.: *Pegomyia pusilla* Mg.

Opius melleus Gahan
U.S.A.: *Rhagoletis mendax* Curran and *R. pomonella* Walsh.

Opius mellipes Prov.
Italy: *Cnephasia incertana* Tr. (Lepidoptera).

Opius nitidulator Nees
Czechoslovakia: *Calliphora vomitoria* L., *Lucilia caesar* L., *Musca domestica* L., *Pegomyia hyoscyami* Panz., *P. hyoscyami* var. *betae* Curt., and *Plusia gamma* L. (Lepidoptera). France: *Chortophila chenopodii* and *Tachina* sp. Britain: *Heliodines roesella* L. (Lepidoptera) and *Pegomyia hyoscyami* Panz. Belgium: *Pegomyia hyoscyami* Panz. and *P. hyoscyami* var. *betae* Curt. Sweden, North America, Germany, Russia and Italy: *Pegomyia hyoscyami* Panz.

Opius obscurator Ratz.
Italy: *Aphis rosae* L. (Hemiptera).

Opius ochrogaster Wesm.
France: *Lithocolletis geniculella* (Lepidoptera).

Opius oscinidis Ashm.
North America: *Phytomyza plantaginis* R.-D.

Opius otiosus Gahan
North America: *Agromyza parvicornis* Lw.

Opius pallidipes Wesm.
Italy: *Acidia caesto* Harr., *A. heraclei* L., *Agromyza macquarti*, *Pegomyia bicolor* Wied., *P. nigritarsis* Zett., and *Tortrix rosana* L. (Lepidoptera). France: *Agromyza macquarti*, *Anthomyia* sp., and *Tephritis* sp. Britain: *Tortrix rosana* L. (Lepidoptera).

Opius pegomyiae Gahan
North America: *Pegomyia hyoscyami* Panz. U.S.A.: *P. vicina*.

Opius perproximus Silv.
Tanganyika: *Ceratitis* sp., *C. cosyra* Wlk. Kenya: *C.* sp., *C. capitata* Wied., *C. cosyra* Walk., and *Dacus* sp. Sierra Leone: *C. giffardi* Bezzi and *C. punctata*. Zanzibar and West Africa: *Dacus ciliatus* Lw.

Opius perproximus modestor Silv.
Kenya: *Ceratitis nigra* Graham.

Opius persulcatus Silv.
Malaya: *Dacus ferrugineus* F. India: *D. incisus* Wlk.

Opius phaeostigma Wlkn.
South Africa and Mauritius: *Dacus ciliatus* Lw. and *D. d'emmerezi* Bezzi.

Opius phorelliae Wlkn.
Transvaal: *Phorellia peringueyi* Bezzi.

Opius polyzonius Wesm.
Europe: *Agromyza albitarsis* Mg. and *A. labiatarum* Hard.

Opius ponerophagus Silv.
India: *Dacus oleae* Gmel.
Opius procerus Wesm.
Europe: *Hylemyia antiqua* Mg. and *Phorbia brassicae* Bch. Sweden: *Pegomyia hyoscyami* Panz. Germany: *P. nigritarsis* Zett.
Opius pumilio Wesm.
France: *Anthomyia verbasci*. Italy: *Pegomyia bicolor* Wied.
Opius pygmaeator Nees
France and Italy: *Anthonomus sorbi* Germ. (Coleoptera).
Opius quebecensis Prov.
North America: *Pegomyia calyptrata* Zett. and *Scaptomyza adusta* Fall.
Opius reconditor Wesm.
France: *Agromyza xylostei* R.-D.
Opius reconditus Wesm.
Italy: *Acidia caesio* Harr. and *Phytomyza xylostei* R.-D.
Opius rhagoleticolus Sachtl.
Germany and Switzerland: *Rhagoletis cerasi* L.
Opius rubriceps Ratz.[1]
Italy: *Magdalis ruficornis* L. and *M. violacea* L. (Coleoptera).
Opius ruficeps Wesm.
France: *Agromyza abiens, Pegomyia conformis*. Germany, Russia and Italy: *Pegomyia hyoscyami* Panz. Belgium: *P. hyoscyami* Panz. and *P. hyoscyami* var. *betae* Curt. Yugoslavia: *Pegomyia nigricornis* Strobl.
Opius rufipes Wesm.
France: *Acidia heraclei* L., *Agromyza mobilis, Lonchaea nigra* Mg., and *Pegomyia acetosa*. Italy: *Agromyza abiens* Zett., *Coleophora corrucipennella* Z. (Lepidoptera), *Domomyza mobilis* Mg., *Elachista griseella* Z. (Lepidoptera), *Lonchaea nigra* Mg., and *Pegomyia hyoscyami* Panz. Britain: *Coleophora nigrocella* Steph. (Lepidoptera).
Opius siculus Monastero
Sicily: *Dacus oleae* Gmel.
Opius spinaciae Thn.
Germany: *Pegomyia betae, P. hyoscyami* Panz. Belgium: *P. hyoscyami* Panz. and *P. hyoscyami* var. *betae* Curt. Sweden, Germany and Holland: *Pegomyia hyoscyami* Panz.
Opius straminator Gour.
Italy: *Orchestes fagi* L. (Coleoptera).
Opius stramineipes Thoms.
Europe: *Agromyza albitarsis* Mg.
Opius striativentris Gahan
U.S.A.: *Phytomyza ilicicola* Lw. and *P. ilicis* Curt.
Opius succineus Gahan
North America: *Agromyza* sp., *A. parvicornis* Lw., and *A. pusilla* Mg.
Opius suturalis Gahan
North America: *Agromyza pusilla* Mg., and *A. scutellata* Fall.
Opius sylvaticus Hal.
Sweden: *Pegomyia hyoscyami* Panz.
Opius testaceus Wesm.
France: *Euphranta conneza* F. and *Gonyglossum wiedmanni* Mg. Italy: *G. wiedmanni* Mg.
Opius tibialis Ashm.
U.S.A.: *Agromyza melanopyga* Lw.

[1] Marshall states that *O. rubriceps* Ratz. should be included in the genus *Cenocoelius*.

Opius trinidadensis Gahan

Trinidad: *Anastrepha serpentina* Wied. and *A. striata* Schin.

Opius utahensis Gahan

North America: *Agromyza parvicornis* Lw.

Opius wesmaeli Hal.

Sweden: *Pegomyia hyoscyami* Panz.

Opius xylostei Marsh.

France: *Phytomyza xylostei* R.-D.

Opius sp.

Hawaii: *Agromyza* sp. U.S.A.: *Agromyza scutellata* Fall., *Phytomyza delphiniae* Frost. Brazil: *Anastrepha fraterculus* Wied. Tanganyika: *Ceratitis* sp., and *Dacus humeralis* Bezzi. Uganda and Zanzibar: *Ceratitis* sp. Sierra Leone: *Ceratitis annonae* Wlk. and *C. coffeae* Bezzi. Kenya: *Ceratitis colae* Silv., *C. nigra* Graham and *C. rubivora* Coq. Korea: *Chlorops oryzae* Mats. Ceylon: *Dacus cucurbitae* Coq. French Cameroons: *Dacus humeralis* Bezzi. Fiji: *Dacus passiflorae* Frogg. and *D. xanthodes* Broun. America: *Eucosma ocellana* F. (Lepidoptera), and *Pegomyia hyoscyami* Panz. India: *Oscinis theae* Big. Germany: *Pegomyia hyoscyami* Panz. Java: *Promecotheca niciferae* Maulik (Coleoptera). Canada: *Rhagoletis pomonella* Walsh.

V. GENERAL BIOLOGY OF *OPIUS ILICIS*

The imagines of *O. ilicis* emerge from the host puparia in the latter half of May and the beginning of June. In 1939, the first specimen to emerge from material collected in the Windlesham area—a male—was observed on 13 May and the last—a female—on the 30th of the same month. Because of the intimate connexion between the development of the *Opius* larvae and the pupation of the host, a point which will be explained more fully in a later paragraph, the emergence of the parasites follows very closely on that of the flies. In the area already mentioned, flies began to emerge on 11 May and continued to come out until the 25th of the month, a period which is only 2–5 days in advance of the *Opius* emergence dates. At Windlesham, females were more common on the whole than males. Out of twenty-three specimens reared from this area in 1939, fifteen were females and eight were males, which would give a sex ratio of 2 ♀♀ to 1 ♂, or 0·5, but the numbers obtained were so small as to make any generalization on this point untrustworthy. Indeed somewhat different figures were obtained in 1937 from a large collection of mined holly leaves, made in the New Forest, Hants. Soon after the arrival of this consignment in Canada flies and parasites began to issue from the mines, and by the end of the emergence period a total of ninety *Opius* adults were obtained. Of these forty-six were females, and forty-four males, so that in this area, and in this season, the sexes were present in about equal numbers.

Mating is a simple affair. No preliminary courtship takes place as in some of the Chalcidoidea and other groups, but the male, when aware of the presence of the female, moves his wings rapidly up and down as if preparing for flight, and then sets off in pursuit. On catching up with her, he mounts rapidly, and copulation, which lasts for only a few seconds, is quickly effected.

Oviposition was not observed, but the following remarks give some indication as to the time when it is most likely to take place. The two possible periods are midsummer (June) and midwinter (probably early December). In June the larva of the leaf-miner is extremely small and is hidden away in the vessels of the midrib, but later in the year, towards the end of November, it moves out into the adjacent mesophyll where it forms a small, but distinctly visible mine (Pl. II, fig. 1). If the female, which in captivity lives for only a few weeks after emergence, attacks the host larva in June, it would, in all probability, do so through the small but distinct oviposition scar made by the fly near the base of the midrib on the underside of the leaf (Pl. II, fig. 3). We must not, however, lose sight of the more likely possibility that it may pass through an alternate host, overwinter as an adult, and then attack the young mines in December, much in the same way as *Chrysocharis gemma*, the other larval parasite of this host, which, after hibernating in the adult stage, oviposits in the fly larvae in the early months of the year. Dissections of holly-fly larvae, in all three stages, made from the time when the mines became visible in early December, until February or March, showed that the parasite was present in the body cavity of its host as a first stage larva. On 7 March, primary larvae were found in newly formed puparia, but no instar other than the first was ever obtained from any stage of the host larva.

The first ecdysis takes place after the host has pupated. From then on development is rapid, and two further ecdyses take place in a very short time. Altogether the parasite passes through four stages, the first being spent in the host larva, where it remains for some considerable time—at least 2, 3, or more months—and the other three in the host pupa, where the total developmental period is about 1 month. The duration of the prepupal and pupal stages, like the larval ones, varies according to weather conditions, but the first usually lasts for about 2 days and the second for 3 weeks.

An extremely interesting phase in the life history of *Opius ilicis* has been observed in the first larval stadium. After the primary larva has attained full size, its growth is retarded for a comparatively long period, and no further development takes place until the host has pupated. Once this has occurred, however, the parasite proceeds to grow very rapidly and reaches larval maturity in quite a short time. Two possible reasons may be put forward to account for this method of development and of these the first is the most likely one, the second in all probability being merely a corollary of the other: (1) it ensures that the maximum amount of food is available to enable the parasite to attain full size as an adult; had the latter completed its development in the host larva, undersized and stunted individuals, if any at all, would in all probability have resulted; (2) it enables the second and third larval instars to feed with the minimum of effort on the easily ingested histolysed tissues of the host. As a result of this method of feeding the cephalic skeleton in these two instars is very poorly developed, indeed it is so reduced that only traces of it can be observed and that with some difficulty.

O. ilicis is an internal solitary parasite of the holly leaf-miner, and although it is very likely that, on occasion, several primary larvae may be found in the same host, only one ever attains to maturity.

VI. DEVELOPMENTAL STAGES

The egg

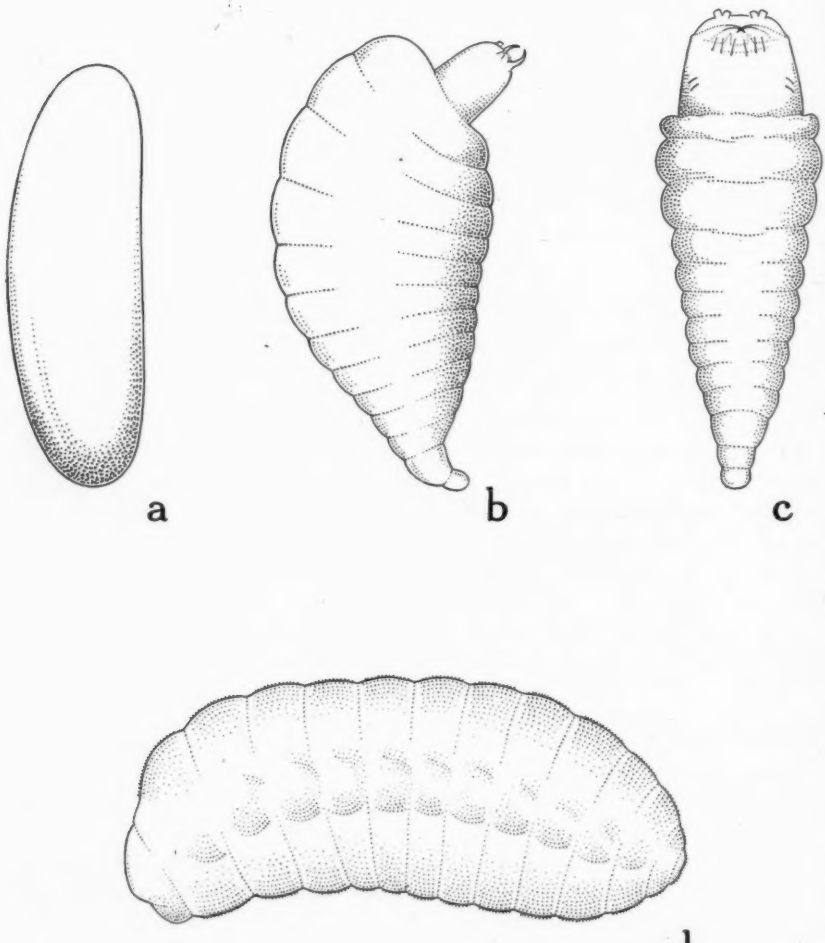
The egg (dissected from a week-old female) is whitish in colour, smooth and kidney-shaped. It is rounded at both ends, one of which is broader than the other and measures 0·2 mm. in length by 0·06 mm. in maximum breadth (Text-fig. 2*a*).

The primary larva

The primary larva of *Opius ilicis* is very typical in appearance and cannot possibly be confused with the corresponding stage of any other parasite of the holly leaf-miner. When viewed from the side (Text-fig. 2*b*) it has a peculiar humped appearance, which is mainly due to the very broad thorax. This unusual shape is somewhat accentuated by the narrow rectangular head and tapering abdomen, the last segment of which is curved round vertically in the form of a "tail". An extremely remarkable characteristic of this larva is its unusual orientation. Contrary to normal experience the concave side of the larva, which would ordinarily be regarded as the ventral surface, is actually the dorsal one. The truth of this can be demonstrated by locating the central nervous system which lies some little distance below the hypodermis on the *convex* (ventral) side of the body. Unless this exceptional curvature is borne in mind, one is liable to misinterpret the nature and position of some of the structures on the head, more particularly the large papillae which would appear to be dorsal antennae but are, in reality, ventral in position and probably correspond to the labial or maxillary palps. Indeed, if this peculiar conformation is general in the primary larvae of the Opiinae, as is very probable, mistakes of this kind have already been committed by certain authors in their descriptions of various Opiine species.

A similar type of orientation to that just described has been observed in the first stage larva of the related Braconid, *Diachasma crawfordi*, by Keilin & Picado (1913). These authors attributed this orientation to an uneven rate of growth, suggesting that the ventral side of the larva had grown more rapidly than the dorsal one. In *Opius ilicis*, and perhaps in other species of *Opius*, it is possible that the serosal cells (mentioned later) on the concave dorsal side may have some connexion with the abnormal curvature of the larva, but whatever the cause or significance of this strange phenomenon may be, it is certainly something very much out of the ordinary, and it cannot fail to be of great value for systematic purposes. Further demonstrations of its existence in other members of the Opiinae will therefore be awaited with much interest.

In dorsal view, the head of the primary larva of *O. ilicis* is quadrate in shape, the thorax broad and the abdomen narrowly tapered (Text-fig. 2*c*). There

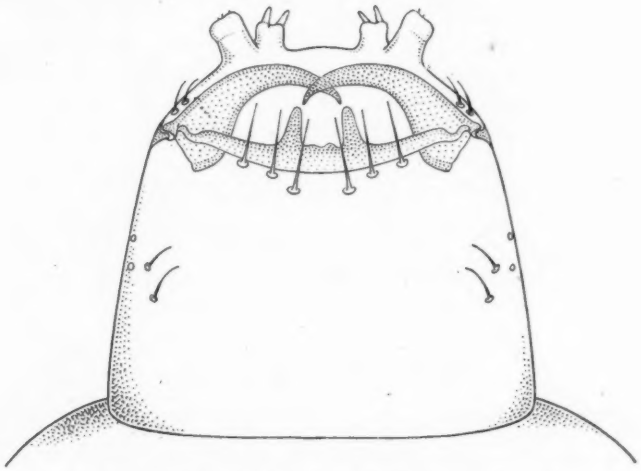


Text-fig. 2. *Opius ilicis*, (*a*) egg (×330); (*b*) primary larva, side view (somewhat contracted); (*c*) primary larva, dorsal view (*b* and *c* × 186); (*d*) mature larva (×46).

are thirteen fairly well-defined body segments in addition to the head and the larva is semi-transparent. In the younger first stage larva a loose mass of large serosal cells with large nuclei is attached to the concave dorsal side of

the body, extending from the head to the posterior part of the abdomen in a similar manner to the serosa in the larvae of *O. humilis* and *O. fletcheri*, described by Pemberton & Willard (1918). These cells are absent in the older primary larvae. When fully grown the first stage larva measures: length 0·40 mm.; breadth at thorax 0·15 mm.; length of head 0·09 mm.; breadth of head at middle 0·09 mm.; length of mandibles 0·05 mm.

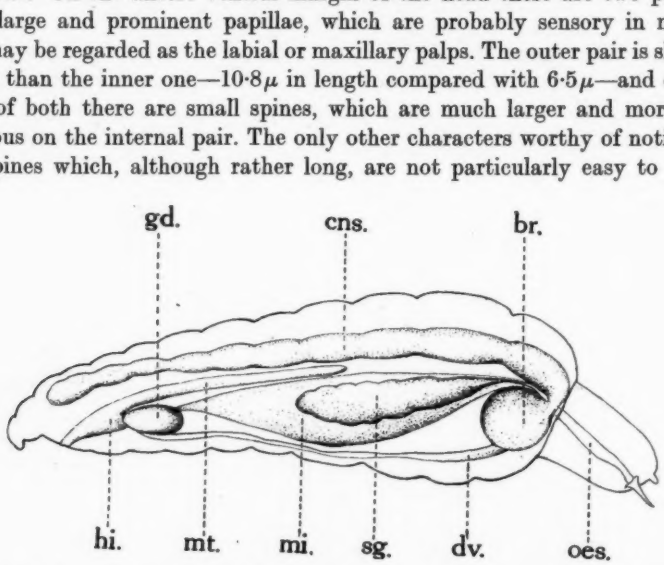
Traces of the two main tracheal trunks partially filled with gas were observed in the body segments, but no spiracles are present in this stage, so that the respiratory system is apneustic, and gaseous exchanges must obviously be effected cutaneously.



Text-fig. 3. *Opius ilicis*, head and cephalic skeleton of primary larva, antero-dorsal aspect (×640).

A close examination of the head (Text-fig. 3) revealed several interesting features, notably the large falcate mandibles, each consisting of a triangular base and a long, curved, well-chitinized tip; the mandibular bar, which name I have applied to the stout rod extending across the head in front of the mandibles in the labroclypeal area; and the large, paired labial or maxillary papillae. Probably the most outstanding structure in the cephalic skeleton of this larva is the mandibular bar, which, as I have already stated, is a thick chitinized rod stretching across the face from one side of the head to the other. It is thicker in the middle and rather tapered towards the ends, and is chiefly remarkable for the very large vertical teeth—7·5μ in length—which project in a U-shaped manner from its centre. It is possible that these teeth are useful for steadying and locking in position the long, curved mandibles when the latter are employed in attacking rival larvae. Sometimes a third, small, and insignificant tooth may be found between the two larger ones, but this

is not invariably present. The superior mandibular strut, pleurostoma and hypostoma are very much reduced in this larva and are represented only by a small pleural sclerite on the wall of the head. This structure, which contains a relatively large socket, affords support to the inner and larger mandibular condyles. On the antero-ventral margin of the head there are two pairs of very large and prominent papillae, which are probably sensory in nature, and may be regarded as the labial or maxillary palps. The outer pair is slightly larger than the inner one—10·8μ in length compared with 6·5μ—and on the ends of both there are small spines, which are much larger and more conspicuous on the internal pair. The only other characters worthy of notice are the spines which, although rather long, are not particularly easy to see in



Text-fig. 4. *Opius ilicis*, side view of primary larva showing internal anatomy (semi-diagrammatic). Note particularly the position of the nerve cord on the *convex* ventral surface which demonstrates the unusual orientation of this larva (×240). *br.* brain; *cns.* central nervous system; *oes.* oesophagus; *dv.* dorsal vessel; *sg.* salivary gland; *mi.* mid-intestine; *mt.* Malpighian tubes; *hi.* hind-intestine; *gd.* gonad.

prepared slides, but can be discerned without much difficulty in physiological salt solution. These are situated in the following positions: two pairs near the mandibular articulation, three pairs in the clypeal region, and four pairs near the outer mid-dorsal margin of the head. A few scattered spines are also present on the body.

The internal anatomy of this larva (Text-fig. 4), because of the abnormal curvature of the head and body, is of more than passing interest. Most important are the positions of the central nervous system and the dorsal vessel. The former lies some little distance beneath the surface on the *convex* side of the larva, whilst the latter occupies a position between the intestine and the body wall on the *concave* side. The nervous system consists of two stout ganglionated longitudinal cords, which stretch from the first to the eleventh body segment. In the thoracic region the ganglia are rather larger than those in the abdomen, whilst the posterior ends of the cords are somewhat

club-shaped in appearance. From each pair of ganglia two stout transverse nerves are given off to supply the various organs of the body. The brain, which is united to the suboesophageal ganglia by a transverse commissure, consists of a pair of large subspherical ganglia situated in the first body segment and not, as is more usual, in the head. It occupies this position probably because of the relatively small size of the latter. The digestive system consists of the mouth, dilatable pharynx, and short oesophagus in the head, the large mid-intestine which occupies the greater part of the body cavity, and a short hind-intestine which terminates at the junction of segments ten and eleven. The hind-gut is often very dilated. An apparently open anus is present (it is possible, of course, that it is covered by a thin almost undiscernible membrane), but there is no open communication between the mid- and hind-intestines. The Malpighian tubes, which arise from the hind-gut are two in number. They extend forward usually to the fourth body segment, but occasionally they are somewhat shorter. A pair of fairly large club-shaped salivary glands are present in the forepart of the body, one on each side of the mid-intestine, extending as far back as the beginning of segment five. Anteriorly they narrow considerably and their respective ducts bend round the dorsal ganglia and unite in the head to form a common salivary duct, which communicates with the mouth. The only other organs of any importance are the gonads, which are two oval bodies situated in the posterior part of the abdomen in the region of the junction of mid- and hind-intestines.

The second and third larval instars

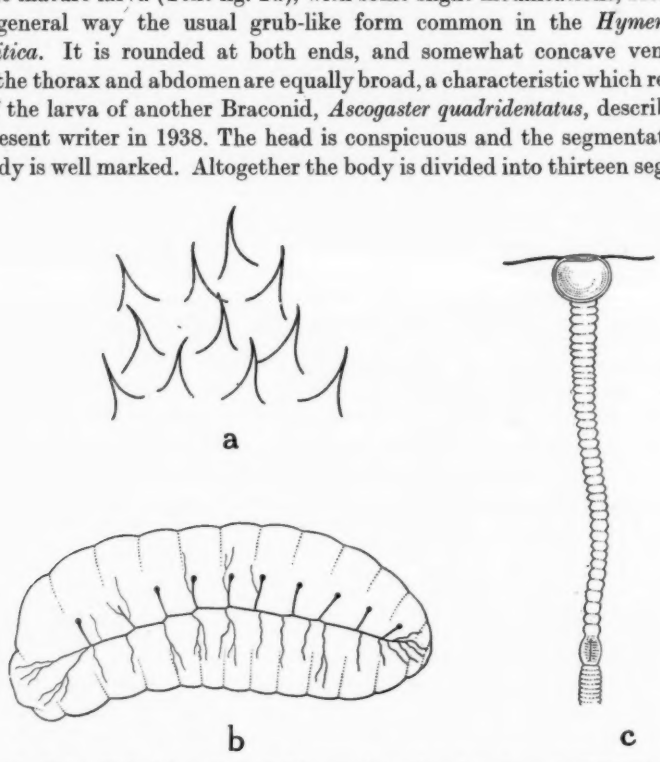
Both second and third stage larvae are characterized by extreme simplification of structure and the absence of chitinized areas. The second instar is somewhat fusiform in shape, with a hemispherical head and fairly well-defined body segmentation. It is for the most part transparent, save for the gut, which is full of brown material. The chief structures on the rather delicate head are two pairs of papillae, a larger outer and a smaller inner pair. The mandibles are present but, being unchitinized, they are extremely difficult to see. They are small with short tips and comparatively large bases. The only other features of any significance are the very large salivary glands which extend into the fourth body segment. Their respective ducts join to form an unusually short and broad common salivary duct just below the mouth. The "degenerate" cephalic skeleton in this and the succeeding instar and its relation to feeding, etc., has already been mentioned, and will be discussed again in a later section. No tracheal system was observed in this stage. The measurements of the second instar larva are as follows: length 0·8 mm.; maximum breadth 0·35 mm.

The third stage larva is very similar in appearance and structure to the second, and there is nothing remarkable to describe. Towards the end of this stage the tracheal system, spines, etc., of the fourth instar may be seen below

the skin. The mature third stage larva measures 1·2 mm. in length by 0·44 mm. in maximum breadth.

The mature larva

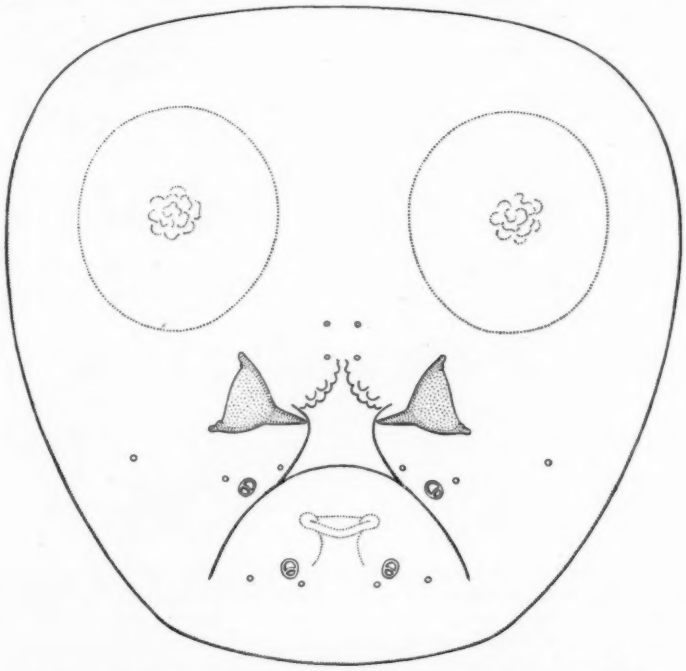
The mature larva (Text-fig. 2*d*), with some slight modifications, resembles in a general way the usual grub-like form common in the *Hymenoptera Parasitica*. It is rounded at both ends, and somewhat concave ventrally, while the thorax and abdomen are equally broad, a characteristic which reminds one of the larva of another Braconid, *Ascogaster quadridentatus*, described by the present writer in 1938. The head is conspicuous and the segmentation of the body is well marked. Altogether the body is divided into thirteen segments



Text-fig. 5. *Opius ilicis*, (*a*) portion of skin armature of mature larva (×825); (*b*) tracheal system of mature larva, side view (×38); (*c*) abdominal spiracle of mature larva (×825).

(excluding the head), the last of which is rather small. In colour the larva is yellowish white, the yellow tinge being imparted by the gorged gut contents. The skin is characterized by a strong, dense armature of triangular spines, which are present all over the body, with the exception of the intersegmental areas. They are absent from the head. These spines (Text-fig. 5*a*), which measure 10μ in height by 7·2μ in diameter at the base, give the larva a decided shagreened appearance and this feature makes the identification of the fourth instar larva a relatively easy matter. A well-developed tracheal system (Text-fig. 5*b*), which is supplied by nine pairs of open spiracles situated on the anterior margins of segments 2 and 4–11, is present in this stage. These spiracles (Text-fig. 5*c*) consist of a rather small, subcircular atrium, 10·5μ

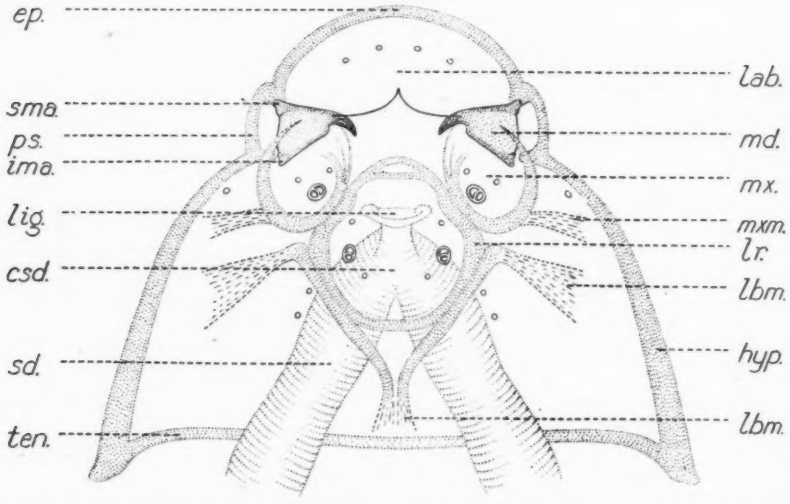
in breadth, 8·6μ in depth, with an external aperture 5μ in diameter, followed by a very long spiracular trachea composed of some 40–50 rings. At the inner end of this trachea there is a small swollen tube, which appears to be a closing device of some sort, similar in construction to that described in the Chalcid, *Sphegigaster flavicornis* (Cameron, 1938), another parasite of *Phytomyza ilicis*. The spiracular tracheae join on to the two main longitudinal trunks which extend through almost the entire length of the body, and are united by transverse commissures in segments 2 and 11. Spiracles are absent from the second thoracic segment, although the spiracular trachea itself is well developed.



Text-fig. 6. *Opius ilicis*, head of mature larva, antero-ventral view (×300).

The head (Text-fig. 6), which is somewhat hemispherical in shape, is characterized by the usual lobes—two upper or epicranial, and one lower, median, or labial. On each epicranial lobe there is a large subcircular antennal area, which is slightly raised in the centre. Around the mouth, the mandibles, labrum, maxillae with slightly chitinized borders, and labium, together with a number of papillae, are the most prominent features. Closer examination reveals a well-developed, though weakly-chitinized, cephalic skeleton (Text-fig. 7). This consists of epistoma, pleurostoma, hypostoma, and tentorium, all unchitinized, a pair of small mandibles with curved chitinized tips, a complete

labial ring, inside which is a chitinized ligula and two **8**-shaped papillae. A pair of similarly shaped papillae are present on the maxillae. The salivary ducts are extremely large, and the common duct formed by their union is very short and broad. In addition to the large "sensory" processes on the labium and maxillae, a number of smaller papillae are present in the following regions: on the labium, two pairs; on the maxillae, two pairs; within the labial ring, two pairs; below the latter, two pairs; and outside the maxillae, one pair; while the lower part of the labium is characterized by a roughened rasp-like structure. In this larva the muscles of the cephalic skeleton are rather well defined, especially those of the mandibles and maxillae, and particularly those of the labium.



Text-fig. 7. *Opius ilicis*, cephalic skeleton of mature larva (×300). *ep.* epistoma; *lab.* labrum; *ps.* pleurostoma; *md.* mandible; *mx.* maxilla; *mxm.* maxillary muscle; *sma.* superior mandibular articulation; *ima.* inferior mandibular articulation; *lr.* labial ring; *lbm.* labial muscles; *hyp.* hypostoma; *lig.* ligula; *csd.* common salivary duct; *sd.* salivary duct; *ten.* tentorium.

The measurements of the mature larva are as follows: length 1·4–2·3 mm., average 1·8 mm.; breadth of thorax and abdomen 0·5–0·8 mm., average 0·7 mm.; breadth of head 0·03 mm.

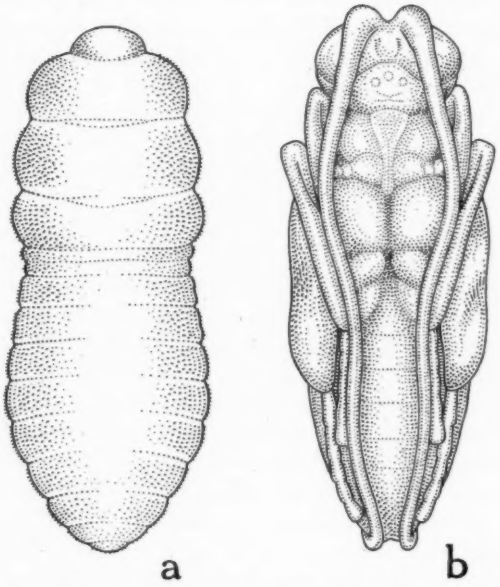
The prepupa

The prepupa (Text-fig. 8*a*), like the mature larva, is characterized by a dense skin armature of triangular spines. It differs from the latter in the constriction of the body into three definite regions: (1) the old larval head with its inconspicuous cephalic skeleton perched on the top of the first body segment—from these two segments, the pupal, and eventually the adult head,

will develop; (2) the thoracic region, consisting of two rather broad segments; and (3) the abdominal region, composed of the ten remaining segments. This instar is whitish grey in colour and its overall measurements are somewhat less than those of the mature larva.

The pupa

When newly formed the pupa (Text-fig. 8*b*) is white in colour, except for the eyes which have a light brown hue. It remains white for quite a long time after pupation has taken place, but towards the end of the pupal period, the



Text-fig. 8. *Opius ilicis*, (*a*) prepupa; (*b*) pupa. Note long antennae. (*a* and *b* ×45.)

head and thorax become black, the abdomen darkens, the wings and antennae change to dark grey, and the legs become straw-coloured. The pupa of *Opius ilicis* is very easily recognized by the long antennae, which extend down over the ventral surface of the body, and curve back over the posterior end on to the dorsum of the abdomen. With this useful character there can be no difficulty in distinguishing it from the pupae of all the other parasites of the holly leaf-miner.

VII. THE COMPARATIVE MORPHOLOGY OF OPIINE LARVAE

As previously indicated, the Opiinae, despite their great economic importance, have received comparatively little attention from systematists. A similar paucity of records exists so far as the biology and larval morphology

of the genus *Opius* is concerned. Probably the best accounts of the latter, to appear thus far, are those of Pemberton & Willard (1918), on *O. humilis* Silvestri, one of the Mediterranean fruit fly parasites, and of Willard (1920), who gave a useful description of the life history of *O. fletcheri* Silvestri, a parasite of the melon fly in Hawaii. The biology of *O. melleus* Gahan, which is parasitic on the blueberry maggot in the U.S.A., has also been very briefly recorded by Lathrop & Newton (1933), but in this case no figures or descriptions of the various larval stages are given. From one or two statements in this paper, however, it appears that the morphology of the latter species corresponds with that of *O. ilicis* in the two following particulars: the long, sharp, curved mandibles of the primary larva, and the absence of conspicuous appendages in the second and third instars. Further comparisons with *O. melleus* cannot be made because of the lack of data provided by these authors. The descriptions of Willard & Pemberton, and Willard, of *O. humilis* and *O. fletcheri* respectively, are very much fuller, but still incomplete. So far as the primary larvae are concerned these two species, like *ilicis*, are noteworthy for their possession of a mandibular bar complete with U-shaped projection. Indeed this feature, which is also present in two species of the related genus *Diachasma*, *D. tryoni* and *D. fullawayi*, would seem to be an extremely useful and general identification mark for primary Opiine larvae. The quadrate head and large falcate mandibles are two further characters which are common to the three species of *Opius* under consideration. Additional points of resemblance between *ilicis* and both *humilis* and *fletcheri* are: the mass of egg serosal cells which are attached to the early primary larva, the apneustic tracheal system and broad thorax of this stage; the apparent absence of tracheae in the second and third instars; the peripneustic tracheal system in the final instar; the well-developed cephalic skeleton of the primary and mature larvae, and the vestigial character of this structure in the two intervening instars; the large body spines on the skin of the mature larva; and, in the pupa, the very long antennae which extend over the full length of the body and curve back dorsally over the abdomen. The main differences between *ilicis* and the other two species are as follows: in the primary larva, (1) the absence of sac-like appendages on the antero-dorsal edge of the first body segment, which are present in both *humilis* and *fletcheri*; (2) the labial or maxillary papillae, of which there are two distinct pairs in *ilicis*, but in *humilis* and *fletcheri*, so far as can be made out from the diagrams, only one pair, whilst in the mature larva of *ilicis* the spines are more generally distributed over the body than in either of the other two species. No detailed descriptions of the cephalic skeleton are given by either of the authors mentioned, but a comparison of Text-fig. 7 in the present paper, and Willard's diagram (1920, p. 429), suggests that there is a labial strut in the mature larva of *O. fletcheri* which is not present in *O. ilicis*. This point, however, owing to the lack of detailed description and labelling of the diagram of *fletcheri*, cannot be stressed.

Further work on the larval morphology of other Opiine species could be undertaken with advantage, but that already done, including the descriptions in the present paper, would seem to indicate that the larvae of this tribe form a fairly homogeneous group. So far as can be ascertained at the moment, recognition of an Opiine larva depends on the following characteristics: in the primary stage (1) the quadrate head; (2) certain features in the cephalic skeleton, particularly the mandibular bar with its U-shaped protuberance; (3) the unusual orientation of the head and body (observed in *O. ilicis* and *Diachasma crawfordi*, but in all probability true also of the other species mentioned, although overlooked by Pemberton & Willard); in the second and third instars (1) the extremely simplified and unchitinized cephalic skeleton; (2) the apparent absence of tracheae; and (3) the lethargic disposition of the larvae: and, in the final instar, the dense armature of spines on the skin of the body. It is also possible that common characters exist in the cephalic skeleton of the mature larva, but further work and more detailed diagrams are necessary before useful comparisons can be made.

VIII. VALUE AS A PARASITE OF *PHYTOMYZA ILICIS*

Although by no means a common parasite of the holly leaf-miner, *Opius ilicis* was found to be present in small but appreciable numbers in most of the areas where collections of mined holly leaves were undertaken. This was particularly true of the Windlesham area where in 1939 its average parasitism was 4%. In certain other districts, however, notably Burnham Beeches, Bucks, where a vast quantity of material was collected, it was very much scarcer. As will be shown in the next part of this paper, *O. ilicis* is intrinsically inferior to *Chrysocharis gemma*, the other larval parasite of the holly leaf-miner, that is to say when the two as larvae come into conflict in the same host, the former succumbs, and the latter usually continues its development. Since this is so, those puparia from which *Opius ilicis* adults emerge represent hosts left unparasitized by *C. gemma*, so that in spite of the intrinsic superiority of the Chalcid, the total mortality in leaf-miner larvae resulting from the combined parasitism of the two species is greater than it would be if the *Chrysocharis* were acting alone. In the hosts where the two parasites conflict, the stronger and more abundant species survives and thus the efficiency of *Chrysocharis gemma*, which is the commonest parasite of *Phytomyza ilicis*, and the one which contributes most to the very high mortality which occurs in this host, is in no way impaired by the presence of its Braconid rival. There can therefore be no question as to the advisability of liberating this species in Canada, for any criticism of its usefulness, on the grounds that it competes adversely with a more efficient parasite, can be countered by the fact, that in all cases where the larvae of the two species were found in the same host, those of *Opius ilicis* were either dead or dying. From these remarks it will be gathered that this parasite, which is responsible for the elimination of a certain

number of hosts escaping the attention of other species, fills a particular niche of its own, and must therefore, even although the percentage of hosts which it accounts for is small, be of some definite value in the scheme of control. It is also possible that it may be even more efficient under the new conditions which exist in the holly areas of western Canada, where the holly leaf-miner is such a troublesome and annoying pest.

IX. Competition with *Chrysocharis gemma*

The intrinsic superiority of the Chalcid *Chrysocharis gemma* over *Opius ilicis* has already been discussed, but the problem of the disposal of excess larvae in one and the same host, which is more difficult of solution, has yet to be examined. In the next part of this paper it is suggested that the large mandibles, mandibular bar teeth, etc., are valuable offensive weapons which enable the dominant larva of *O. ilicis* to emerge successfully from intraspecific combats. When the conflict is of an interspecific nature, however, and the primary larva of this species is involved in a fight with the corresponding stage of *Chrysocharis gemma*, the latter usually proves victorious. It is true that, owing to the comparative scarcity of hosts parasitized by *Opius ilicis*, the number of dissections carried out to prove this statement was somewhat limited, but nevertheless all actually undertaken pointed to the decided superiority of the Chalcid. When one considers the apparently superior armament and larger size of the latter (length of mandibles in *Opius* 0·05 mm., do. *Chrysocharis* 0·02 mm.; length of *Opius* larva 0·40 mm., do. *Chrysocharis* 0·30 mm.; maximum breadth of *Opius* larva 0·15 mm., do. *Chrysocharis* 0·08 mm.) this result is rather surprising. The method of elimination employed by the *Chrysocharis* parasite, judging by the injured and melanized Opiine larvae observed, is direct frontal attack. In one instance a host was found to contain an active primary larva of *Chrysocharis gemma* and a dead first stage *Opius* larva. The latter had its skin punctured both in the thoracic and abdominal regions. Significantly enough, these punctures were paired, suggesting that they had been caused by the opposing mandibles of the victorious Chalcid, whilst the areas surrounding the punctures were melanized, the dark colour probably being brought about by the oxidation of albuminoid substances in the blood and the precipitation of uranidine (*vide* Imms (1934), p. 135).

Two possible reasons may be put forward to account for the marked inferiority of the Braconid: (1) despite its larger size, it is obviously very much less active than its wiry opponent, and this lethargic disposition may be attributed to two causes, (i) it is in a kind of diapause pending the transformation of the host into the pupal stage; (ii) it is older, and apparently less energetic than the more recently emerged and "metamorphically" vigorous *Chrysocharis*, and (2) it is possible that it may be partially paralysed as a result of the poison injected into the host by the Chalcid female prior to oviposition, and is thus less able to withstand the onslaught of its competitor.

This latter possibility will be more fully discussed in a moment. Although it would appear that the normal method of disposal employed by *C. gemma* is direct mandibular attack, this is not the only way in which the supernumerary larvae are eliminated. On one occasion a host was found to contain an active *Chrysocharis* larva and a dead first instar *Opius*. The latter bore no trace of melanized punctures or injury of any sort, and it seems quite possible that this larva owed its death to the poison injected by the *Chrysocharis* female. At any rate a comparison of the effects produced after parasitization by each species suggests that this result may sometimes occur. When a host is attacked by *C. gemma* it very quickly changes from an active, turgid, bright, shiny, lemon-coloured larva to one which is paralysed, flaccid, and of a pale dirty yellow hue. A *Phytomyza* larva parasitized by *Opius ilicis*, on the other hand, or at least one containing a primary larva of this species, remains active, and no evident signs of parasitization are apparent. If the poison injected by the *Chrysocharis* female is powerful enough to induce such a revolutionary change in the character of the host, it would appear equally possible for some sort of adverse effect to be produced on any *Opius* larva which may be present in the body cavity of a host subjected to this treatment. In certain instances it may be that death results, and in others the parasite may be paralysed and weakened to such an extent, that despite its apparently superior equipment, it becomes quite unable to compete successfully with its Chalcid rival.

It has been suggested by Spencer (1926), in the case of certain Aphid parasites, that all but one of the competing larvae are killed by a biochemical process of inhibition rather than by direct mechanical injury. This explanation is supported by Thompson & Parker (1930), who state that: "It is more probable as Spencer has suggested in the similar case of *Aphidius*, that at a certain moment, soon after hatching, the larvae begin to pour into the blood a cytolytic enzyme which affects the tissues of the host, and those of the younger larvae of *Eulimneria* itself." Whether this is the final solution of the problem or not, is not yet certain, but at any rate we can say, with some measure of assurance, that although mechanical injury would appear to be one method by which supernumerary larvae are eliminated, it is by no means the only one.

X. SOME GENERAL CONSIDERATIONS

A number of interesting points which have a general bearing on the study of parasite larvae will be discussed in this section in the following order:

(i) The function of the cephalic skeleton in successive instars.

(ii) A note on the taxonomic value of the cephalic skeleton in the parasitic Hymenoptera.

(iii) Respiration and the development of the tracheal system in *Opius ilicis*.

(iv) Arrested development in the Opiinae and some related forms.

(i) *The function of the cephalic skeleton in successive instars*

The composition of the cephalic skeleton in the four larval instars of *O. ilicis* has been described in some detail in Part VI. All that remains to be done here is to relate this composition and degree of development to function. In the first and fourth instars the system of facial rods is well developed, but in the second and third it has become so reduced as to be almost non-existent. The following reasons are now put forward to account to some extent for this marked divergence in development. In the first stage larva, the cephalic skeleton, with its massive mandibles and paired teeth (Text-fig. 3), would appear to be developed almost exclusively for offensive purposes. It is also possible that the mandibles may be of some use in tearing up portions of the fat body, but their main function seems to be the destruction of rival larvae. Since *O. ilicis* is a comparatively rare parasite of the holly leaf-miner, no examples of hosts containing more than one larva of this species were obtained, but in the common allied species, *O. fletcheri*, Willard (1920) noted that in hosts with more than one primary larva of this parasite, a struggle for survival took place. "Many cases have been observed", he states, "where there were only one living and from two to eight dead parasite larvae in the same host individual. This struggle takes place immediately after hatching and usually within four hours, all but one of the larvae of *Opius fletcheri* have been killed." In another species, *O. melleus*, described by Lathrop & Newton (1933), a similar destruction of excess larvae has been observed. It is therefore highly probable that the strong well-developed cephalic skeleton in the first stage larva of *Opius* is primarily designed for aggressive action.

In the second and third instars the cephalic skeleton has been so reduced that it is almost invisible. This extreme reduction would seem to be correlated with the feeding habits of these two stages. In the histolysed tissues of the host, food is available in a highly assimilable form, and in addition there are no rivals to fight at this period of the life history, because *Chrysocharis gemma* does not attack the puparium, and the pupal parasites *Sphegigaster flavicornis* and *Chrysocharis syma*, etc., have not yet appeared on the scene. There is therefore no apparent necessity for the facial rods to be highly developed in either the second or the third instars, and their presence at all, even in a reduced form, can be explained only on the grounds that the mandibles are of some use in directing or wafting particles of food into the mouth (any breaking up of particles which may take place is almost certainly accomplished by the chemical action of the saliva, and in this connexion the presence of extremely large salivary glands and broad ducts in these larvae is noteworthy), and that they provide a foundation on which the well-developed skeleton of the fourth instar may be built up.

In the mature larva the cephalic skeleton (Text-fig. 7), although not particularly robust, is nevertheless well developed. Its function in this stage appears to be the cleaning up of the more solid tissues which remain after all

the liquid nutriment has been absorbed. As no cocoon is formed, its development in this species can have no relation to spinning activities, etc. The marked development of the labial area in this instar is interesting from a systematic point of view, and will be discussed at some length in the following subdivision.

(ii) *A note on the taxonomic value of the cephalic skeleton in the parasitic Hymenoptera*

It has been pointed out in previous papers by the present writer (1938, 1939), that the cephalic skeleton, among other anatomical features, affords some very useful characters for the separation of species and of larger systematic groups. A comparison of this structure in representative parasites of the holly leaf-miner, *Opius ilicis*, for the Braconidae and *Chrysocharis gemma* and *Sphegigaster flavicornis* for the two Chalcid families, Eulophidae and Pteromalidae respectively, should therefore prove to be of general taxonomic interest. The most striking differences between these two classes occur in the mature larvae, but the earlier stages also provide points of distinction. In the primary instar the Braconid differs from the Chalcids: (1) in its peculiar orientation (*vide* p. 20); (2) in the possession of larger and more falcate mandibles (0·05 mm. in length, cf. 0·02 mm. in *Chrysocharis gemma*); (3) in the possession of a mandibular bar with two prominent teeth, which is not represented in the corresponding area of either *Chrysocharis* or *Sphegigaster*, unless this rod is considered to be equivalent to the epistoma; and (4) in the possession of a small pleural sclerite on the wall of the head (Text-fig. 3), and the absence of a hypostoma, which structure is present in the two Chalcid species.

In the intermediate instars the cephalic skeleton is fairly well developed in the Chalcids, but vestigial in the Braconid.

The main points of difference between the two groups in the mature larvae are to be found in the labial and maxillary areas. In the labial area of *Opius*, as in most Braconid and Icheumonid larvae, there is a circular sclerite which has been termed the labial ring (Text-fig. 7), a structure which is absent from the cephalic skeleton of the Chalcid representatives. Within the labial ring there are two **8**-shaped processes which may possibly be sensory in nature. Some writers have considered them to be the analogues of the labial palpi of the adult, but this interpretation is somewhat questionable. A similar pair is present on the maxillae. These papillae, as well as the labial ring, are entirely wanting in the Chalcids. Probably the best distinguishing mark of Braconid and Ichneumonid larvae, as pointed out by Thompson (1930), is the presence (with certain exceptions) of these two structures, the labial ring and the labial and maxillary sensillae, while the absence of them affords a useful clue to the identification of the immature stages of the Chalcidoidea. Other distinctive marks in the cephalic skeleton, of a more subsidiary character, are the presence in the Ichneumonidae and Braconidae of labial and maxillary

struts, and a ligula, and their absence in the Chalcidoidea. Sometimes the mandibles of Braconid larvae are toothed, a character which is often a useful guide to this group, but in *Opius*, and probably in most of the smaller members of the family, they are simple and unpectinated.

(iii) *Respiration and the development of the tracheal system in* O. ilicis

In *O. ilicis* the tracheal system, instead of following the more usual type of development, is rudimentary in the primary larva (traces of the two main tracheal trunks partially filled with gas have been observed in the body segments), highly developed in the final instar (Text-fig. 5*b*), and apparently wanting in the two intervening stages. It is possible, of course, that fluid-filled tracheae are present in these larvae, but this is a point which could be ascertained only by sectioning. The first three instars, despite the apparent absence of tracheae in the second and third, must obviously obtain the necessary oxygen by cutaneous respiration, that is, through the skin, by a process of diffusion from the blood of the host, whilst the fourth obtains it in a more direct manner through the spiracles. The main point of interest in the respiration of *O. ilicis* is the absence of any sort of functional tracheal system in the second and third instar larvae. It might be argued that if a supply of oxygen in the tracheae was necessary in the first-stage larva, it would be even more necessary in the larger second and third instars. However, if we correlate respiration with activity we will see that this unusual method of development of the tracheal system is quite in keeping with the character of the larva. In the first stadium the larva has a pair of very large and powerful mandibles to wield, and this action when combined with other aggressive movements calls for a good deal of muscular activity, which, in turn, is dependent on an ample supply of oxygen—hence the presence of gas in the tracheae. (The nature of this gas has already been discussed by the present writer when dealing with the parasites of the pea moth (1938, pp. 295–6).) In the second and third instars there is no fighting to be done, and the only apparent activity, apart from some slight movement, is connected with digestion and allied functional processes. Since food is present in a highly assimilable form, a minimum amount of oxygen will be used up during the course of its conversion, and this low metabolic activity, when taken in conjunction with the extremely lethargic nature of these two stages, is sufficient to account for the absence of functional tracheae in the second and third instars.

The tracheal system of the mature larva is of the normal type and therefore no comments on its functioning are necessary.

(iv) *Arrested development in the Opiinae and some related forms*

The temporary cessation of growth which takes place in the larval development of *Opius ilicis* towards the end of the first stadium and the dependence of this parasite on certain changes in the host before further growth can take

place have been mentioned in section V, but these phenomena are so interesting and offer such a marked contrast to the mode of development of *Chrysocharis gemma*, the other larval parasite of *Phytomyza ilicis*, that they are worthy of closer attention. The occurrence of a similar break in development in the following species: *Opius humilis*, *Diachasma Tryoni*, *D. fullawayi* (Pemberton & Willard, 1918); *Opius fletcheri* (Willard, 1920); *O. melleus* (Lathrop & Newton, 1933); and *Dacnusa areolaris* (Haviland, 1922), confirms the observations made on *Opius ilicis* and renders the subject still more interesting. All the parasites mentioned above, with the exception of the last named, which is a member of the closely related tribe Dacnusidae, are Opiines, so it is quite possible that further work on other members of the group will reveal arrested development to be a normal proceeding in this section of the Braconidae. A somewhat similar occurrence has been observed in the Ichneumonid—*Dioctes punctoria* (Thompson & Parker, 1928), a parasite of the European corn borer. This species overwinters in the corn-borer caterpillar as a primary larva, and further development does not take place until the spring, a method of hibernation which is totally different from that of the morphologically indistinguishable larva of *Eulimneria crassifemur*, another parasite of this host. The latter completes its metamorphosis in the late autumn and overwinters as a full-grown larva within its cocoon. Although similar in some respects, this arrest in development in *Dioctes* differs in at least three important particulars from that of *Opius ilicis* and its allies: (1) the Ichneumonid has a mature host at its disposal, whereas the larva in which *O. ilicis* lives continues to develop throughout the winter months; (2) in *Dioctes* there appears to be an absence of any dependence on the pupation of the host for the initiation of further growth in the spring, which is such a marked peculiarity of the Braconid; and (3) the phenomenon in the Ichneumonid cited, as in many other species with a similar habit of development, appears to be related solely to hibernation, which is definitely not the case in the Opiines. According to Dr W. R. Thompson, further examples of arrested development, of a type similar to that of *Dioctes*, are to be found in the Tachinidae, particularly in the Melanophorine parasites of woodlice, which, like *Dexia rustica* on *Melolontha melolontha* and *Masicera senilis* and *Zenillia roseanae* on *Pyrausta nubilalis*, hibernate in the host in stage II, and also in *Zygobothria nidicola* (Howard & Fiske, *Bull. U.S. Dept. Agric.* 91, p. 225), which overwinters in stage I, in the young caterpillars of the brown-tail moth.

In view of the fact that the Chalcid *Chrysocharis gemma* attacks the same stage of the holly leaf-miner as *Opius ilicis*, and like the latter is endoparasitic on this host, it will be interesting to compare the larval development of the two species. The first ecdysis does not take place in *Opius* until the host has pupated, but in *Chrysocharis*, on the other hand, larval development proceeds unhindered, and appears to be quite independent of the size or age of the host. As a result there is a good deal of variation in the size of the adult parasites which emerge from the leaf-miner larvae, much larger imagines being produced

from the mature hosts than from the younger stages. Haviland (1922) has observed similar variation in another Chalcid and has suggested that this diversity of size, owing to variability of nutrition, may lead to far-reaching results, such as for example, the production of new strains within a particular species. Keilin (1915) also commented on size variation in the Tachinid *Pollenia rudis*. He pointed out that the size of the imago was determined by the dimensions of its host, and stated that if the size difference should make mating impossible between large and small forms, distinct races might arise within the species. A good example of size variation in parasite adults has been observed in the Ichneumonid *Pimpla turionellae*. Three females, of which two were very much larger than the third, were reared by Mr A. L. Abel of this Laboratory, the two larger ones from the pupae of *Tortrix postvittana* and the smaller one from the puparium of *Actia pilipennis* (Tachinid), a parasite of this Tortricid. The first two were more than twice as large as the third—11 mm. in length (including ovipositor) compared with 4·5 mm. Mating between individuals of such diverse sizes would appear to be impossible, and as a result of this incompatibility three eventualities may be envisaged: (1) the small females mating with small males may produce a distinct race of small individuals parasitizing the Tachinid larvae only; (2) the small imagines may be unable to find suitable mates and so die out (this is unlikely); or (3) the small females when mated may lay directly in *postvittana* larvae so that in the next generation normal individuals may again be produced and thus the occurrence of undersized individuals, being purely accidental, would not materially affect the history of the species, except in so far as it reduced its potential efficiency. Of these three possibilities, the first and the third are the most likely to occur. Of course, in the foregoing example the matter is a little more complicated, because of the hyperparasitic tendency of the Pimpline, but the main issue—the relation of size to nutrition—remains the same. Many more examples of size diversity could be brought forward, but this one must suffice. In the case of *Chrysocharis gemma* no special observations on the relation between age and size of host and the ultimate size of the adult parasites have been made, but the subject, given the necessary time, would be an interesting one. It is perfectly obvious, however, that although this Chalcid can develop on hosts of varying sizes and ages, the Braconid *Opius ilicis*, for some reason or other, is apparently unable to do so, and hosts in an advanced stage of development appear to be absolutely necessary for the completion of its metamorphosis. In discussing this problem, the most important point to bear in mind is the extremely close relation which exists between host pupation and the initiation of the second stage of growth in the parasite. How this latter is brought about may best be answered by suggesting that it is dependent on the production of certain chemical or physical changes in the composition of the host. That vast changes, both of a chemical and physical character, do take place at the time of pupation is well known, but the discovery of the exact nature of the exciting cause must await future

physiological experiments. Another question that is extremely difficult to answer, because of the very great, if not insurmountable obstacles lying in the path of experimentation on such small insects, is—what advantage has this particular mode of development for *Opius ilicis* and its allies? In reply, the following speculation may perhaps be permitted, but it must be clearly understood that it is only an expression of opinion for which, at the moment, because of the nature of the problem, there is no definite proof. It is possible that the main significance of arrested development in *O. ilicis* and related Braconid species is, that it is a method for ensuring the conservation of the food supply until it is sufficiently large to produce a normal-sized imago. Why this should be necessary in one species and not in another is not quite clear, unless it be that the metabolism of this parasite is of such a nature that the production of any adults, apart from stunted and probably useless individuals, demands the maximum amount of food for the proper development of the larvae. One beneficial result, which follows this developmental hiatus, however, is quite apparent, and that is, that the adults of *O. ilicis*, unlike those of *Chrysocharis gemma*, are all, more or less, uniform in size.

XI. SUMMARY

1. While investigating the parasites of the holly leaf-miner (*Phytomyza ilicis* Curt.) with a view to utilizing them in the control of this troublesome pest of holly in western Canada, a species of *Opius*, which on examination proved to be new to science, was reared from the fly puparia.

2. A fairly complete account of the general systematics, distribution, biology, and morphology of the various developmental stages of this parasite is set down in the preceding pages. The primary larva is particularly interesting because of its unusual orientation. After the anatomical details had been worked out it was discovered that the concave side of the larva, which would normally be regarded as the ventral surface, is actually the dorsal one.

3. The genus *Opius*, whose distribution is world-wide, contains a very large number of species which parasitize important economic pests. In temperate regions the insects which suffer most from their attacks are species of *Pegomyia*, *Agromyza*, *Rhagoletis*, *Phytomyza* and *Cerodonta*, whilst in tropical and subtropical areas the most favoured hosts belong to one or other of the two genera *Dacus* and *Anastrepha*.

4. The host relationship of the genus, because of its importance from both economic and taxonomic standpoints, is discussed at some length.

5. In the first stadium *Opius ilicis* is a larval parasite, but the three succeeding instars live in the host pupa, and the imago emerges from the puparium. A very interesting phase in the life history of this parasite occurs towards the end of the first stage. At this point the development of the larva is arrested and further growth cannot take place until the host has pupated.

6. Very little work has so far been carried out on the larval morphology of the Opiinae, but that done up to the present, including the foregoing

descriptions, would seem to indicate that the larvae of this tribe form a fairly homogeneous group. The main distinguishing characters of these larvae are listed in section VII of this paper.

7. It is pointed out that *O. ilicis*, in spite of being intrinsically inferior to *Chrysocharis gemma*, is responsible for the destruction of a certain number of hosts which escape the attentions of the latter parasite, and although the percentage accounted for is small (maximum parasitism in 1939 4%), it nevertheless fills a particular niche of its own, and so must be of some definite value in the scheme of control.

8. The chief method employed by the first instar of *Chrysocharis gemma* in the destruction of rival *Opius* larvae would appear to be direct mandibular attack. Several reasons have been put forward to account for the decided inferiority which is exhibited by the Braconid when it comes into conflict with this Chalcid.

9. In section X, a number of interesting points which have a general bearing on the study of parasite larvae are discussed. These include the cephalic skeleton and its probable function in successive instars, the taxonomic value of this structure in the parasitic Hymenoptera, the apparent absence of a tracheal system in the second and third instar larvae of *O. ilicis*, and arrested development in the Opiinae and some related forms.

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Fig. 1

Fig 2

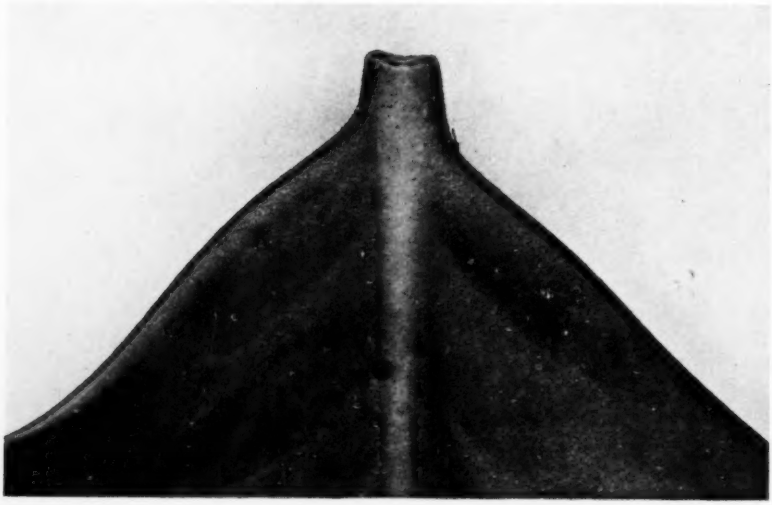

Fig. 3

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EXPLANATION OF PLATE II

Fig. 1. Holly leaf with incipient mine of *Phytomyza ilicis*. From mines of this size, which are present on the trees in December, the primary larva of *Opius ilicis* has been dissected out.

Fig. 2. Holly leaf with mature mine of *P. ilicis*. From this mine a pupal parasite, such as *O. ilicis*, has emerged.

Fig. 3. Underside of holly leaf showing two oviposition scars of *Phytomyza ilicis*. Through these scars, as suggested in the text, the females of *O. ilicis* might be able to parasitize the very young leaf-miner larvae.

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THE INFLUENCE OF NUTRITION ON EGG-PRODUCTION AND LONGEVITY IN UNMATED FEMALE BODY-LICE (*PEDICULUS HUMANUS CORPORIS*: ANOPLURA)

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(With 1 Figure in the Text)

Introduction

The object of the present experiment was to determine to what extent the longevity and yield of eggs of female body-lice is affected by the length of time allowed daily for feeding. The main difficulty has been to ensure uniformity of conditions and to make the feeding period, as far as possible, the only variable.

Bacot (1917) pointed out that female lice will lay eggs before fertilization and in the continued absence of males, though these infertile eggs do not hatch. His findings have been amply confirmed. Buxton (1937) has shown that the presence of males in large numbers may have a detrimental effect on females, decreasing both their reproductive activity and their longevity. This is apparently due to repeated copulation and to the injuries—sometimes fatal—which may be inflicted during this rather violent act. For these reasons it was decided that only unmated females would be used in the experiment. This provision also eliminates such possible influences on reproductive activity as early death of the male.

The lice were reared and kept in small pillboxes floored with bolting silk. These were worn under the sock as suggested by Buxton (1939). The insects were isolated in the last larval stage to prevent the possibility of fertilization, which may occur within the few hours following the last moult. To secure uniformity it was necessary to wear the lice continuously and on no occasion was a female removed from the body for more than 20 min. each day—the time required for washing and for counting the eggs. The day was divided into 4 hr. periods and groups of ten females were fed for 24, 20, 16, 12, 8 and 4 hr. per day respectively. Another group of ten was never fed after the last moult had taken place. To prevent feeding at other times, the boxes were placed, in the intervals, in slightly larger half-pillboxes. The floors of these were of double thickness and were perforated by several holes of 5 mm. diameter which allowed air to circulate between the skin and the interior of the box containing the louse. Thus the only essential difference between feeding period and interval was that during the latter the gauze floor of the box was raised sufficiently from the skin surface to prevent the louse biting. The feeding period was always a single unbroken stretch. The time at which it started was

varied a little from day to day in case some activity of the host, taking place at a fixed hour daily, might influence the lice. In no case was a batch made up of females which had emerged on the same day. This provided that, should a particular day prove unusually favourable or otherwise, its influence would not be brought to bear on all members of a group at the same period of life. The boxes were all worn under the socks but their positions were changed daily in case one area might be better than another. All the lice were fed throughout adult life on a single host—the writer—and the great majority were reared from the egg or first instar on the same host and under the same conditions as the adults.

Marsh & Buxton (1937) and Mellanby (1932) have demonstrated the striking uniformity of temperature and humidity existing between the clothes and the trunk under varying atmospheric conditions. The present experiment thus approaches controlled conditions, these unmated lice being confined singly throughout their adult life in identical boxes, always in communication with this very stable atmosphere next to the skin and always on the same host. The experiment took place during the very cold weather of December–January 1939–40 and in practice it was suspected that the temperature under the socks did fluctuate somewhat as, on one or two extremely cold days, the egg production of all groups was diminished.

The eggs were counted and removed daily, each louse being given a fresh piece of black tape for oviposition. Eggs were rarely laid on the box itself but were frequently deposited on the gauze. This was particularly common during the first few days of adult life in all groups and throughout life in the case of underfed groups.

The group of ten was found to be rather small, a single louse being able to sway the final results for the batch to some extent, but was necessitated by the fact that there is a limit to the number of lice which can be tolerated over a long period.

RESULTS

General

The results for each louse are given in Table 1, the individuals of each group being arranged in order of longevity. Group totals, means and standard deviations are given in Table 2.

The normal pre-oviposition period was 1–2 days in all groups. In six individuals only (all from batches fed 12 hr. or over) was it prolonged to 3 days. The full rate of production is not attained till the fifth or sixth day. From this time on the rate is maintained till shortly before death. As a rule no eggs are laid on the last day of life but occasionally numbers up to four were recorded. In view of the statement made by Nuttall (1917) that high rates in comparison with previous figures might be attained if lice were worn continuously, it is interesting to note that in the 24 hr. group 200–300 eggs per louse was a common figure, 329 being the highest recorded. Very high rates of laying

Table 1. *Records of longevity, egg yield and rates of laying, of individual lice (a, b, etc.)*

Hours...	24			20			16			12			8			4		
Ref. no.	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day	Days life	Eggs	Eggs/day
a	6	11	1·8	5	10	2·0	20	133	6·7	7	13	1·9	3	3	1·0	4	3	0·8
b	17	121	7·1	5	12	2·4	23	148	6·4	19	114	6·0	4	2	0·5	4	3	0·8
c	30	228	7·6	30	218	7·3	26	169	6·5	26	175	6·7	4	2	0·5	5	2	0·4
d	30	246	8·2	30	243	8·1	29	199	6·9	28	178	6·4	4	2	0·5	9	23	2·6
e	32	263	8·2	31	254	8·2	29	203	7·0	29	199	6·9	5	9	1·8	9	29	3·2
f	33	220	6·7	33	174	5·3	30	198	6·6	29	207	7·1	5	10	2·0	10	20	2·0
g	36	282	7·8	33	258	7·8	35	246	7·0	31	220	7·1	21	99	4·7	10	33	3·3
h	36	296	8·1	35	238	6·8	37	249	6·7	34	239	7·0	22	89	4·0	18	61	3·4
i	38	329	8·7	38	262	6·9	41	296	7·2	35	227	6·5	30	169	5·6	28	123	4·3
j	40	310	7·8	42	171	4·1	44	270	6·1	38	264	7·0	36	196	4·3	36	189	5·6

Table 2. *Group totals, means and standard deviations. The latter are calculated with a correction for small samples* (Hill, 1937, p. 46)

	Longevity (days)			Egg yield			Eggs/louse/day	
Hours	Total	Mean	S.D.	Total	Mean	S.D.	Mean	S.D.
24	298	29·8	10·4	2306	230·6	96·6	7·7	2·0
20	282	28·2	12·7	1840	184·0	96·3	6·5	2·3
16	314	31·4	7·7	2111	211·1	53·1	6·7	0·3
12	276	27·6	8·9	1836	183·6	72·4	6·7	1·6
8	134	13·4	12·6	581	58·1	75·1	4·3	3·7
4	133	13·3	10·8	486	48·6	60·0	3·7	1·7

were maintained for short periods by single lice—in the 24 hr. group it was quite common for a louse to lay ten eggs per day for 4 or 5 days in succession. Eleven eggs in a day was quite a common figure among members of the 12–24 hr. groups and the 20 and 24 hr. groups sometimes reached the figure of twelve. In the 24 hr. group 13 eggs were laid in 1 day on two occasions. It was noted, however, that except in the 20 and 24 hr. groups these very high figures are usually followed by a fall on the subsequent day. It appears probable that, for practical purposes, ten eggs per day is the highest rate that a louse can be expected to maintain. The highest rate recorded in the present experiment was 8·7 eggs per day for entire life or 9·4 for full reproductive life (i.e. sixth to penultimate day inclusive).

In the 12–24 hr. groups old age was usually the apparent cause of death. Two died of rupture of the gut. In one case (20 hr. group, *j*) the oviduct became blocked. This louse, though tensely swollen and unable to lay, lived in this condition for almost a fortnight and is largely responsible for the rather low results obtained in this group. In the 4 and 8 hr. groups the "individual temperament"—the faculty of seizing every opportunity for feeding—became important. Most of the members of these groups died early. Usually one feed was missed and by the time the next was due the louse was too weak to take

the opportunity. This was tested in the case of a few females not included in the experiment. It was found that if these were taken from their boxes in this moribund condition they might often be induced to feed on the wrist by breathing on them gently for a few minutes. If a feed were taken they immediately returned to normal and might live thereafter for a considerable time, but if left to themselves they invariably died. In deaths of this type it is thought that dehydration is at least as important as starvation.

It was noticed that at the final moult quite a large amount of blood might be retained in the gut. The unfed group was started in order to determine whether this blood, along with accumulated food reserve, was sufficient to permit of egg-laying before death. The females of this batch were chosen to include some very large and some very small lice but all died on the third day without laying. Accordingly, this group is omitted from the calculations, except those concerned with longevity.

Longevity

Some difficulty was met in assessing the significance of the figures. The samples are too small for a straightforward application of the standard error test of the difference between means (Hill, 1937, p. 57). The method finally employed was to work out a combined standard deviation for the two groups to be compared and subsequently to determine whether the two means could reasonably be expected to occur within the same universe. As a check, the group totals were also subjected to the test for goodness of fit by χ^2. In every case the results of these two tests were in agreement and the results appear reasonable.

It seems probable that no significant difference in longevity occurs between the 12, 16, 20 and 24 hr. groups, nor between the 4 and 8 hr. groups. Significant differences exist between the unfed and 4 hr. groups and between the 8 and 12 hr. groups. These three main subdivisions are well shown in the histogram (Fig. 1*a*). Thus unfed lice died very early—3 days—and lice fed 4 and 8 hr. daily also had rather short lives, averaging about 13 days. Those fed 12 hr. and over lived, on an average, about 30 days. The longest recorded life was 44 days, a figure which falls far below the maximum of 61 days noted by Buxton (1939).

Rates of laying

The same procedure was adopted in considering the rates of laying and the same conclusions were reached. Again no significant difference was detected between the 12, 16, 20 and 24 hr. groups nor between the 4 and 8 hr. groups. The difference between the 8 and 12 hr. groups was found to be significant (Fig. 1*b*).

It was thought that if only the full reproductive life were considered (from, say, the sixth to the penultimate day inclusive) more evenly graded results might be obtained. This procedure eliminates many lice which died

in the first week, before reaching their full reproductive capacity. The results, however, followed rather closely the same trend as before, the rates being 8·8, 7·6, 7·7, 7·7, 5·9 and 5·2 eggs per louse per day in the 24 to 4 hr. groups respectively. Such being the case, it has seemed preferable to use the entire figures and the whole length of life. The initial lag and the occurrence of early deaths are, after all, part of the general picture.

If in each group every separate day's lay by each louse is noted and if the number of times each figure occurs is summed, the mode (or most frequently occurring number) may be found. If these results are expressed as percentages (Table 3) a more accurate basis for comparison between groups is gained.

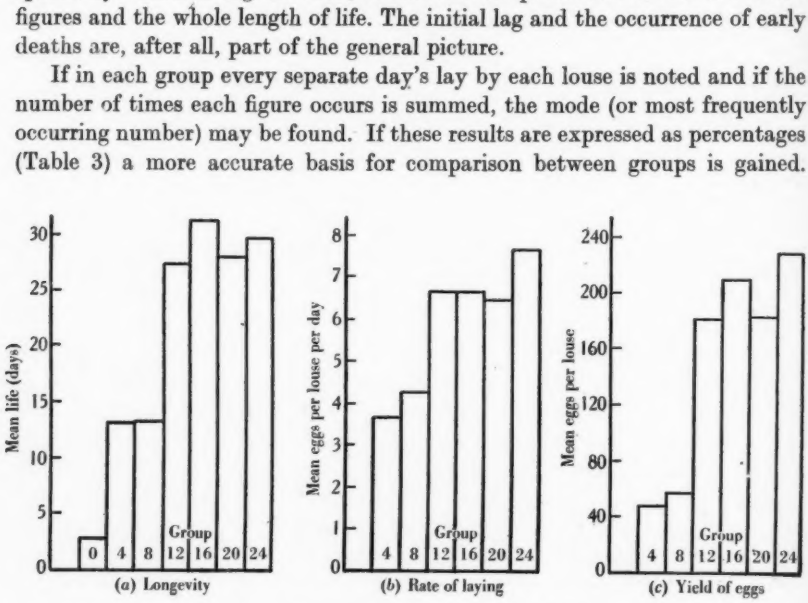

Fig. 1. Mean longevity, rate of laying and yield of eggs. All groups.

Table 3. *Percentage frequency with which different numbers of eggs were laid per day. The mode is in italics: days on which an insect laid no eggs have been omitted in calculating it*

	Number of eggs													
Hours	0	1	2	3	4	5	6	7	8	9	10	11	12	13
24	8·4	1·3	2·4	1·0	2·0	3·0	4·7	7·0	15·4	21·2	*21·5*	8·4	3·0	0·7
20	13·4	2·8	2·1	1·8	5·0	5·7	8·9	9·6	*14·9*	*14·9*	13·8	5·3	1·8	—
16	10·5	1·0	1·9	2·6	3·8	4·1	8·9	12·7	*23·9*	18·1	9·9	2·6	—	—
12	7·6	3·6	0·7	1·5	1·5	7·2	13·8	*19·9*	*19·9*	13·4	8·0	2·9	—	—
8	20·2	2·2	2·2	6·7	13·4	*17·2*	15·8	11·2	6·7	3·7	0·7	—	—	—
4	19·5	8·3	4·5	11·3	11·3	*18·0*	14·3	10·5	0·8	1·5	—	—	—	—

Except for calculation of percentages the days on which an insect laid no eggs are neglected. It is seen that in the series the mode reaches a higher figure progressively with increase in feeding time. The sequence is fairly regular and discloses a serial effect not shown by the means of the groups. Thus, according to the mode the groups present an ascending series instead of two main subdivisions.

Eggs per louse

The figures for eggs per louse show within each group a very wide scatter as they take no account of the length of life. They afford a poor basis for discussion in comparison with the rates of egg-laying. It is thought that, in view of the small size of the samples and the scatter of the results, little reliance can be placed on statistical tests of significance. Such were indeed applied, but without consistent results. A consideration of the histogram (Fig. 1*c*) shows that the results are comparable with those of the preceding section. Thus the 4 and 8 hr. groups may be regarded as similar, as may the 12–24 hr. groups. Possibly the 24 hr. group may be segregated from the 12 to 20 hr. groups, but the evidence is inconclusive.

CONCLUSION

It is concluded that until the feeding period is reduced to less than 12 hr. per day little decrease occurs in longevity or reproductive activity. Below this level a sharp fall occurs in both. In general, the rates of egg-laying were high, as compared with others that have been recorded (Buxton, 1939, p. 36); the suggested practical application is that where large stocks of lice are being reared the rate of production may be increased by keeping them close to the skin even when they are not feeding. This would appear to be preferable to keeping them in the pocket or in an incubator. Further, in the case of persons who find it injurious or impossible to permit continuous feeding, the feeding period may be reduced to 12 hr. per day without seriously lowering the output of eggs, so long as the boxes are worn constantly under the clothes.

SUMMARY

1. Isolated unmated female body-lice were worn in pillboxes between the skin and the clothes. They were kept constantly on the body but, by a simple device, groups of ten were permitted feeding periods of different length. These groups were fed for 4, 8, 12, 16, 20 and 24 hr. per day respectively. Another group of ten were never allowed to feed after the last moult.

2. Some of the figures for egg yield were high. Lice in the 24 hr. group were able to maintain a rate of ten eggs per day for 4–5 days at a time.

3. No significant difference in longevity or rate of egg-laying was found to exist between the 12, 16, 20 and 24 hr. groups nor between the 4 and 8 hr. groups but a pronounced and significant difference exists between the 8 and 12 hr. groups. Below 12 hr. there is a sharp fall in longevity and rate of egg production. The unfed group all died, without laying, on the third day.

4. The rate of laying as shown by the mode increases progressively with increase in time allowed daily for feeding.

5. With regard to the mean eggs per louse the position is less clear. It is felt that the 24 hr. group may differ significantly from the 12, 16 and 20 hr. groups but this is uncertain.

I have pleasure in thanking Prof. P. A. Buxton and Dr C. G. Johnson for the constant help they have given me with statistical problems and for the useful criticism to which they have subjected the results. I wish also to thank the laboratory staff of the Entomology Department, London School of Hygiene and Tropical Medicine, for advice on many technical points concerned in the rearing and maintenance of the necessary stock.

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ON THE SEARCH FOR HOSTS AND THE EGG DISTRIBUTION OF SOME CHALCID PARASITES OF THE KNAPWEED GALL-FLY

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(With 3 Figures in the Text)

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I. Introduction

The object of this paper is to present data which show how certain parasitic Hymenoptera distribute their progeny among their hosts under natural conditions. The results are examined in the light of certain current mathematical theories of animal populations and of parasite behaviour, with a view to discovering how far they support the primary assumptions on which these theories are based.

A mathematical theory is a description of the relationships between things in nature, which, for the sake of convenience, is greatly simplified. The type of formulation is often more to be regarded as a mathematical convenience than as an indication that the factors represented have exactly the relationship postulated. It is not to be expected therefore that a mathematical theory will be accurate; and it is particularly desirable to know how inaccurate the primary assumptions may be in specific cases.

One common assumption in mathematical theories is that certain processes proceed in a random manner. Confusion has arisen in the literature through failure to notice that the term random can be used in many different ways. In this paper the term will be used as follows:

Random distribution. Fiske (1910) used this term in a numerical sense, and it will be used here in this way unless it is stated that the term refers to a random distribution in space. If a parasite distributed its eggs at random amongst its hosts the number of hosts (y) which contain p parasites should, according to Stoy (in Salt, 1934), be

$$y = \frac{1}{p!}\left(\frac{x}{N}\right)^p N e^{-x/N}, \qquad (1)$$

where N is the number of hosts, x the number of parasite eggs distributed, and e the natural logarithmic base (2·7183).

Random search. This term was used by Nicholson (1933) and Nicholson & Bailey (1935) in their theory of balance of animal populations. They give no precise definition. Their use of the term shows that in random search the number of encounters between predator and prey, or parasite and host, is proportional to the product of the population densities of the two species. The term will be used here in this numerical sense. However, a random search would be expected to lead to a distribution of parasitism which was random in space.

Random movements. Laing (1937, p. 315) states that "a parasite finds its host, as any animal must find the things it seeks, by movements which, until they are influenced by the qualities of the object sought, are random with respect to it. When the qualities are perceived they direct the movement, which thereupon ceases to be random." It must be noted that if parasites search for their hosts by random movements, this does not necessarily imply that parasitism will be distributed at random either numerically or spatially. For a random search all hosts must be equally available. If search is by random movements those hosts far from the parasite are less available than those close to it. As random movements continue, and as more parasites search over the same area, parasitism will become more and more evenly distributed, over the area considered, and the effects of the search will approximate more and more closely to those of random search.

The parasites discussed in this paper all attack the same host, the common Trypetid fly *Euribia jaceana* Hering, known until recently as *Urophora solstitialis* L. in this country (Collin, 1937). All the data, unless otherwise stated, were collected from a series of square metre plots all within 70 yards of one another along a strip of uncultivated land extending on either side of a cart track at the edge of the University Farm, about 3 miles west of Cambridge. The census area was to all intents and purposes undisturbed during the period of study, except in so far as the collection and removal of material for study was concerned. This amounted to less than 10% of the total knapweed in any season.

The life history of the host has already been described (Varley, 1937*a*). The flies lay their eggs in small groups within the flower heads of knapweed, *Centaurea nemoralis* Jord., a subspecies of *C. nigra* L., in July, some weeks

before the flowers bloom. The eggs hatch in 9–12 days, depending on the temperature, and the newly hatched larvae, already in their second instar, enter separate florets and burrow down till they reach the ovules, which swell up and form flask-shaped woody galls instead of fruits. Usually more than one larva is present in a flower head, and the galled fruits coalesce to form a single multilocular gall in which each larva occupies a separate cell, within which it feeds head downwards and becomes fully grown in August. In the following May the larva reverses its position in the cell, and pupates facing the exit. The adult emerges in July.

II. THE EGG DISTRIBUTION OF THE PARASITE *EURYTOMA CURTA*

A brief description of the life history of this species, together with illustrations of the egg and larval stages, has been given elsewhere (Varley, 1937*b*). The egg of *Eurytoma curta* Walk. is laid in small second instar larvae of *Euribia jaceana*. The egg soon hatches, but the resulting larva grows very slowly at first, and the host becomes fully grown at about the normal time. Then the host is caused to pupate prematurely (see Varley & Butler, 1933). It turns around in its cell, and forms its puparium; but at this period the contained parasite larva begins to grow very rapidly, and the host is consumed within a few days. Within the host puparium *Eurytoma curta* completes its development, pupates in the following summer, and emerges as an adult usually in the month of July.

Field observations have established some interesting facts about the way in which the female *Eurytoma* seek their hosts under natural conditions. Females normally fly quite slowly, and can be followed by eye without much difficulty. When they reach a flower head of knapweed they hover round it, and either pass on or settle on it and explore the bracts with the antennae. They may then leave the flower head almost at once, or insert the ovipositor one or more times, and remain on the flower head for half an hour or more.

A female was watched on 8 August 1936 for 19 min., and each flower head with which it made contact was taken to the laboratory and dissected under the microscope. The female was first found with its ovipositor pushed into a flower head, and it left 2 min. later. During the next 20 sec. it settled on two flower heads and left them. The fourth flower head was examined for $2\frac{1}{4}$ min. before the ovipositor was inserted, and the fly left 11 min. later. In the next 3 min. four more flower heads were examined, and the female then flew away and hovered in front of three other flower heads before it was lost to sight. None of the flower heads that had been examined contained any hosts. There was no apparent difference between those flower heads into which the fly had inserted its ovipositor and those it had rejected after a short examination with the antennae.

On 20 July five females were found examining flower heads and two of these were using the ovipositor. No suitable hosts were found in any of these

flower heads; though one of them contained two eggs of *Euribia jaceana* which were nearly ready to hatch, the parasite on this head had not used its ovipositor in the search. On five other occasions *Eurytoma curta* females were found on flower heads; two of the flower heads contained unparasitized hosts, and two of the flower heads were without hosts. In the fifth case the parasite was found with the ovipositor inserted, and two out of the four second instar hosts contained eggs of *E. curta*.

It was clear that the female parasites recognized the flower heads as objects of special significance. In flight they often hovered close to flower heads, or settled on them. They neither visited other plants to any extent, nor did they pay any attention to the leaves or stems of the knapweed. The flower heads are probably recognized by sight, though it is quite possible that smell plays some part also. The presence of hosts within flower heads is not detected by the preliminary examination with the antennae, but hosts are in fact sought with the ovipositor.

(i) *The distribution of progeny—superparasitism*

Many hundred hosts were dissected, yet only on one occasion was superparasitism observed, when one live and one dead larva of *Eurytoma curta* were found within the same host. How many cases of superparasitism would have been expected if the distribution of eggs had been random? In 1935 there were dissected 538 hosts in the second or early third instars, and there is little doubt that had more than one parasite been present in them this would have been noticed; 226 hosts were parasitized, one of them contained two parasites and the rest only one egg or larva. The frequency distribution actually observed can be compared in two different ways with that expected if distribution were random.

First we can calculate the expected frequency if the parasites had distributed their 227 eggs amongst the 538 hosts at random, using formula (1), the results of which are given in Table 1 A. We see that had this number of

Table 1. *Egg distribution of* Eurytoma curta

	No. of parasites per host						Total eggs
	0	1	2	3	4	5	
Frequency of hosts found	312	225	1	0	0	0	227
Calculated frequencies of hosts: A	353	149	31·4	4·4	0·5	0	227
B	312	170	46·3	8·4	1·1	0·1	293

eggs been distributed at random only 34% instead of 42% of the hosts would have been parasitized, and that some thirty-six cases of superparasitism would have occurred. This method of comparison is the one which has been used by Salt (1934); but if, as seems probable, the factor which limits the success of the parasite is the difficulty of discovering hosts, it is preferable to compare the results with the hypothetical situation which would arise if the parasites discovered the same fraction of the hosts, namely, 42%. Using again Stoy's

formula we find that the parasite would need to lay 293 eggs at random to attain 42% parasitism, and that fifty-three cases of superparasitism would be expected to occur. The calculated frequency distribution is given in Table 1 B. By this method of comparison we see that if the *Eurytoma* females did in fact search for their hosts at random they must have encountered hosts some 293 times, and that sixty-seven of these hosts had already been encountered previously, and had been parasitized. In only one case out of these sixty-seven was a second egg laid. This indicates the very high degree of discrimination shown by *E. curta*.

These results are confirmed by data collected in 1936, when 243 hosts were dissected; sixty-eight of them contained either a single egg or larva of *E. curta*, and none contained more than one.

Since the only part of the parasite which comes into contact with the host is the ovipositor, it must be by senses resident in this organ that distinction is made between parasitized and unparasitized hosts. Salt (1937) proved that *Trichogramma* avoids superparasitism by such means, and Fulton (1933) has described small sense organs on the tip of the ovipositor of *Habrocytus cerealellae*.

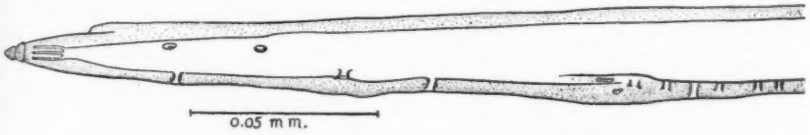

Fig. 1. Tip of ventral valve of the ovipositor of *Eurytoma curta*.

In *Eurytoma curta* the median dorsal valve of the ovipositor seems to lack sense organs, but the paired ventral valves (Fig. 1) each have three organs at the very tip, which appear as slender tubes leading from the end of the lumen. Close to the tip are five other sense organs which appear as circular pits in the integument. Then there is a long series of about thirty sensilli (only six of which are shown in Fig. 1) extending in a line towards the base of the ovipositor. The ovipositors of various other parasitic Hymenoptera have been examined, and all have sense organs of some kind near to the tip and also along the ovipositor itself.

(ii) *The influence of uneven host distribution on parasitism*

Smith (1939) recognizes the theoretical importance of the effect of local host concentrations on the efficacy of a parasite's search. Here field observations provide some information on this rather neglected aspect of parasitism. In 1935, when both hosts and parasites were numerous, some 20 sq. m. of ground were cleared of knapweed between 13 August and 15 October. The last eggs of *Eurytoma curta* were observed on the former date, so it may be assumed that the attack of the parasites was complete when this series of samples was collected. All the hosts were dissected, so that parasitism would have been

detected if it had occurred, unless, as was frequently the case, the host was completely destroyed by some other parasite, or by the predacious larva of the common moth *Eucosma scopoleana*.

The data can be examined in two ways. We can analyse numerically the data which refer to flower heads containing different numbers of hosts, and find the frequency distribution of parasitism in them. In order to avoid error here data are only included from those flower heads in which no hosts had been destroyed by other parasites or caterpillars.[1] Secondly, we can analyse the spatial distribution of parasitism in different square metre samples, and find if the parasitism is correlated with host density, which should not be the case if distribution were purely random.

(1) *Parasitism in flower heads containing different numbers of hosts.* The data are given in Table 2 and Fig. 2. The first row of figures gives the frequency

Table 2. *The parasitism of* Euribia jaceana *by* Eurytoma curta *in flower heads containing different numbers of hosts*

	No. of hosts in flower head									
	1	2	3	4	5	6	7	8	9	14
Frequency	317	351	246	127	64	24	11	5	1	1
Frequency with parasites	133	197	158	93	47	20	8	4	1	1
% flower heads attacked	42	56	68	72	72	87	73	80	100	100
Standard error	2·8	2·6	2·8	3·9	5·5	7·6	13·5	18	—	—
Calculated % flower heads attacked	40	60	70	75	78	79	79	80	80	80
Total hosts	317	702	738	508	320	144	77	40	9	14
Total parasites	133	278	295	193	128	56	18	15	5	11
% parasitism	42	40	40	38	40	39	23	38	55	80
Standard error	2·8	1·8	1·8	2·2	2·8	4·1	4·8	7·7	17	18

with which flower heads with different numbers of hosts were found, and the second row gives the number of these flower heads which contained one or more parasites. From these figures the percentage of flower heads attacked successfully by the parasites is derived, and given in the next row; the standard error (σ) of the percentages ($100\,a/b$) is estimated by the formula

$$\sigma = \frac{100}{b}\sqrt{\frac{a\,(b-a)}{b}}.$$

The percentage of parasitism remains remarkably constant at about 40%. Only one value out of ten is just significantly different from the value for single hosts; and as the level of significance taken was 1 in 20 this might well be due to chance. On the other hand, the percentage of flower heads attacked by the parasites rises with increasing numbers of hosts in the flower heads, and the differences are strongly significant. At first sight this might be due to the

[1] This precaution was not necessary in the analysis of the spatial distribution of parasitism; thus the percentage of parasitism in the two sets of data does not correspond. The true percentage of parasitism was just over 40%; but *Eurytoma curta* was found in only 29% of the total gall cells.

parasites being attracted to those flower heads in which hosts are concentrated; but field observations rule out this possibility. The parasites discover flower heads while in flight, and do not confine their search with the ovipositor to flower heads containing hosts, but stab apparently at random in flower heads which may or may not contain hosts. Now in a random search with the ovipositor, the greater the number of hosts in the flower head the greater will be the chance that one at least will be found by the parasite. The curve *A* in Fig. 2 is formed on this basis (see legend) and fits the observed points reasonably well. It is enough to conclude here that the data are in agreement with the view that the search for hosts takes place in two stages, each of which is random.

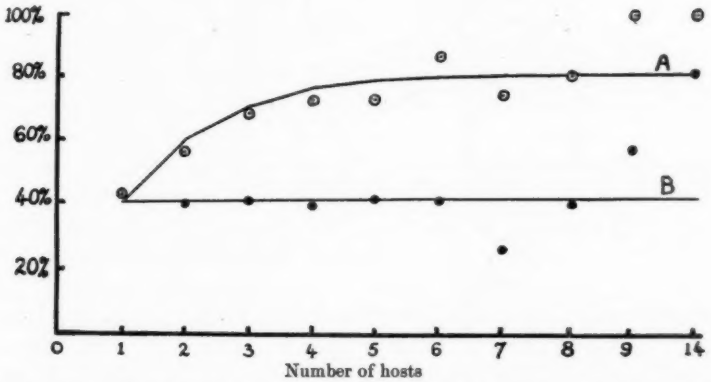

Fig. 2. The parasitism of the gall-fly larvae by *Eurytoma curta* in flower heads containing different numbers of hosts. ⊙ Percentage of flower heads attacked. The curve *A* was calculated on the following assumptions: The parasites discovered only 80% of the flower heads, and searched in these with the ovipositor by random stabbing movements. The chance that any single host was discovered in such a flower head was taken to be 0·5. ● Percentage of hosts parasitized, with the line *B* drawn at 40% parasitism.

(2) *Analysis of local variations in host and parasite density.* Table 3 gives the census data from a series of twenty samples of knapweed gathered at Madingley between 13 August and 15 October 1935. During this period 22 sq. m. were sampled, but two of them contained fewer than thirty hosts, and were rejected because percentages based on such small numbers are not reliable.

The object of this analysis is to see if the distribution of the hosts and parasites differs significantly from that which would be expected if it were purely random. First, we can find if the host distributed its progeny at random, and secondly, we can study the distribution of the parasites amongst the hosts. The methods used are those described by Fisher (1925).

(*a*) Using the data in Table 3 the correlation coefficient between the number of flower heads per square metre and the number of hosts per square

Table 3. *Census data showing the distribution of* Euribia jaceana *(the host) and* Eurytoma curta, *its parasite, in* 20 *sq. m. plots at Madingley in* 1935

Plot no.	No. of flower heads	No. of heads containing hosts	No. of hosts	No. of parasites
1	430	94	208	73
2	390	112	254	105
3	224	63	172	66
4	236	33	86	19
5	306	54	102	26
6	405	84	191	45
7	200	47	94	31
8	270	61	145	49
9	436	42	99	21
10	332	135	422	159
11	383	130	300	107
12	264	98	267	92
13	133	54	147	54
14	207	53	119	47
15	164	68	171	68
16	79	27	68	25
17	88	13	37	15
18	136	31	90	32
19	73	17	36	9
20	225	83	203	80
	4981	1299	3211	1123

metre was calculated and found to be $r = +0{\cdot}54$. This is a significant correlation, as shown by calculating $t = 2{\cdot}68$, for which the probability $P =$ about 0·015. This correlation is fairly strong, and shows that the host density is to a considerable degree dependent on the amount of available environment. But we can find if it is in fact strictly dependent on this by finding the correlation coefficient between the number of flower heads per square metre and either the number of hosts per flower head, or the fraction of flower heads which contain hosts. These work out to be $r = -0{\cdot}2$ and $r = -0{\cdot}02$. Neither is significant; but the slight negative correlation between the number of flower heads and the number of hosts per flower head indicates that the hosts laid rather more eggs in places where flower heads are few than would be expected. But this tendency is so slight that the correlation is not significant, and must be regarded as unproven.

(*b*) Using the same data an attempt was made to see if the percentage of parasitism of the hosts by *Eurytoma curta* was correlated with host density, in terms of hosts per square metre. The correlation coefficient worked out to be $+0{\cdot}322$, which is not significant. If, however, we use the number of hosts per flower head, that is, the density of the hosts in relation to the available environment, there is a strongly significant positive correlation and $r = 0{\cdot}607$.

In view of the fact that in an earlier section of this paper it was shown that the percentage of parasitism in flower heads containing one, two, three and more hosts was always about 40% this result is rather surprising, and apparently contradictory, especially when it is remembered that it is by the analysis of the same body of data in two different ways that these results have been obtained. However, as will be seen, this apparent contradiction can be re-

solved by recourse to the method of partial correlation. By this technique if a number of variables a, b, c, d are correlated together by the correlation coefficients r_{ab}, r_{ac}, etc., then we can calculate the "partial correlation" between a and b by eliminating the effects of c and d. To this partial correlation coefficient the symbol $r_{ab.cd}$ is attached. The partial correlation coefficient indicates the correlation to be expected were the eliminated variables constant.

Using this method the partial correlation coefficients between the percentage of parasitism (a), the number of hosts per square metre (b) and the number of hosts per flower head (c) were calculated. We have seen already that $r_{ab} = +0{\cdot}322$, and $r_{ac} = +0{\cdot}607$; and we find that $r_{ab.c} = -0{\cdot}08$ and $r_{ac.b} = +0{\cdot}554$. The first of these is quite insignificant, showing that there is no correlation between the percentage parasitism and the number of hosts per square metre if the effect of the number of hosts per flower head is eliminated. On the other hand, the correlation between the percentage of parasitism and the number of hosts per flower head remains strongly significant on eliminating the effect of the number of hosts per square metre.

So far then the apparent contradiction remains; however, the number of hosts per flower head can be split up into two variables, the mean number of hosts per galled flower head (d) and the percentage of galled heads (e). We now require the partial correlation coefficients $r_{ad.be}$ and $r_{ae.bd}$, which work out to be $r_{ad.be} = 0{\cdot}317$ and $r_{ae.bd} = 0{\cdot}564$. The former does not reach the $P = 0{\cdot}1$ level of significance, while for the second P is less than 0·01, and the correlation is strongly significant. We see then that the percentage of parasitism is correlated mainly with the variable (e), the percentage of galled heads, and that there is no significant correlation with the number of hosts per gall. This result then is after all in agreement with the conclusion reached on p. 52 that the percentage of parasitism is independent of the number of hosts in a gall. Having established that there is a significant partial correlation between the percentage of parasitism by *E. curta* and the percentage of flower heads which contain galls we may calculate the regression of the first of these variables on the second. This regression coefficient works out to be 0·43, which means that a unit rise in the percentage of flower heads which contain galls is on the average accompanied by a rise of 0·43, in the percentage of parasitism. In Fig. 3 the regression curve is plotted and the actual points obtained from the census of each square metre are superimposed upon this to form a spot diagram.

(iii) *Discussion of searching by* Eurytoma curta

The strongly significant correlation between the percentage of parasitism by *E. curta* and the local variations in the fraction of flower heads containing hosts shows that search is not entirely random in space. The field observations of the search for hosts by this species enable some tentative conclusions to be reached as to the origin of the correlation. We have seen that the parasites

did not apparently recognize flower heads which contained hosts by external examination, but only after probing with the ovipositor. This shows that the parasites must be quite unable to sense an area in which the proportion of host-containing flower heads is high. The concentration of the attack by the parasites in such areas cannot be due to any reaction prior to the discovery of a host, but can only be due to some reaction taking place after the discovery of a host. As a parasite passes through a series of areas in which the proportion of galled flower heads varies, the probability is that the first host discovered will be in an area in which the proportion of galled heads is high. It is tentatively suggested that the correlation arises as a result of a changed searching behaviour after the discovery of a host, and that in this changed type of searching the immediate neighbourhood of the host is searched with special thoroughness. Such a change in behaviour is known in *Trichogramma* from the work of Laing (1937).

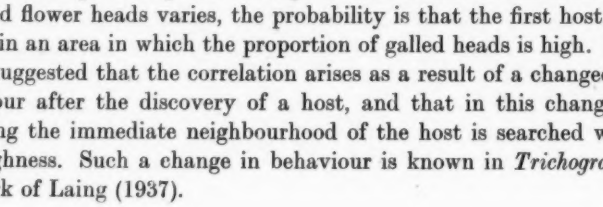

Fig. 3. Correlation between the percentage of parasitism by *Eurytoma curta* and the percentage of flower heads containing hosts, in 20 sq. m. samples, with calculated regression curve.

The high percentage of parasitism in areas of high host density indicates that the parasites must spend a longer time searching in such areas of relative host abundance. Thus the effective host density, and the chance of encountering hosts will be somewhat above what would be the case were all areas searched equally. The rate at which hosts are discovered is governed largely by the local host density, because we have seen that the hosts are actually found by the slow process of probing with the ovipositor, often in flower heads which contain no hosts. In the square metre plots examined the percentage of flower heads with hosts varied from 10 to 41, with a mean of 27, while the percentage of parasitism ranged from 21 to 41. If the size of the samples does not greatly reduce the apparent variation in host density, any tendency to confine the search to areas of high host density could at most increase the efficiency of the search by 50%.

III. THE EGG DISTRIBUTION OF THE ECTOPARASITIC SPECIES

The life histories of *Eurytoma robusta* Mayr, *Habrocytus trypetae* Thoms. and *Torymus cyanimus* Boh. have been briefly described, and the egg and larval stages have been illustrated by Varley (1937*b*). The life history of the fourth parasite dealt with here, *Eupelmella vesicularis* Retz., has been described by Morris (1938). All of them lay their eggs on the outside of the larvae of their hosts, or perhaps within a puparium upon some suitable host within, and one host can support only a single parasite larva. The eggs are all easily recognizable. That of *Eurytoma robusta* is dark brown with a long tail: that of *Habrocytus trypetae* is partly covered with minute papillae: the egg of *Torymus cyanimus* is smooth, and lacks any tail; while that of *Eupelmella vesicularis* is smooth with a short tail, and is invariably covered with a little silky pad. When the eggs are freshly laid there is no difficulty in making an accurate count of their number; but usually they had hatched before the count was made, and only the shells were left. In most cases these were easily found, and could be identified and counted accurately, but in some cases when a well-grown larva of a parasite was present no trace of the egg shell was discoverable. This was usually the case later on when the parasite had become adult and had emerged. The egg shells then stood a fair chance of being covered up by the meconium of the larva, or of being destroyed when the adult bit its way out of the gall, and enlarged the narrow passage to the outside world. Allowance has to be made for these errors. They can conveniently be divided into two types, which are fairly distinct from one another:

(1) If the failure to discover eggs which were present was purely random, then the error introduced would be negligible, because the shape of the frequency distribution would be unaltered.

(2) If all the eggs in a batch escaped discovery, then a correction can be applied to minimize the error involved.

These cases belong to some group other than the 0-group. They were therefore omitted from the frequency distributions, and the 0-group was reduced in proportion. This correction has been applied to all the cases where no eggs were found on a parasitized host. There is a possibility that this may in fact lead to overcorrection, as errors of a random kind might lead to the same result. This will tend to *decrease* the apparent superparasitism.

(i) Eurytoma robusta

The occurrence of this parasite was very local. No trace of it had been discovered in the large amount of preliminary census work which was made on knapweed collected from many different localities in a number of different counties. At Madingley it was found first in the fresh flower heads in July 1935, when a few eggs and young larvae were seen. Its distribution in the various square metres was very irregular. In 23 sq. m. collected in 1935 after

the first eggs were seen, over half of the eighty-three hosts attacked were in 3 sq. m.; in 1936 its localization was even greater, for 20 sq. m. were examined after the time when the parasites should have appeared, and thirty-two out of thirty-eight parasitized hosts were in a single square metre. The information about the egg distribution of such an uncommon species as this is necessarily scanty, but the data from 1 sq. m. in which the species was common in 1936 are sufficient to bear further consideration. The figures obtained from the census are given in Table 4, and their agreement with the values calculated on the

Table 4. *Egg distribution of* Eurytoma robusta

	No. of eggs on a host					
	0	1	2	3	4	5
Observed frequency of hosts	48	26	2	3	0	1
Calculated frequency	46	25·3	7·0	1·3	0·2	0·02

assumption that within this 1 sq. m. the eggs were distributed singly and at random is very close (lumping groups 3–5, $\chi^2 = 3{\cdot}9$, $n = 2$ and P is between 0·1 and 0·2). Within this very limited area, then, the parasite behaved as if it laid its eggs at random, but since all the other square metres examined contained few or no eggs of this species, the whole distribution was not random, but erred considerably on the side of excessive superparasitism, which in 1936 caused 27% mortality.

Apparently only a small number of females of *E. robusta* were active in the census area; within the particular square metre in which most hosts were attacked, the data support the idea that hosts are sought at random; but the areas reached by the different females did not overlap, and most of the area was quite free from the attentions of the species.

This is a very clear instance of the way in which search by random movements may lead to a distribution of parasite progeny which is not random when the parasite density is very low.

(ii) Habrocytus trypetae

This species has two or three generations in the year, depending on the warmth of the summer. The hosts become available as soon as they are well grown in August, and some adult *Habrocytus* may emerge from these galls in September, and lay more eggs on the surviving hosts. The over-wintering larvae of *Habrocytus* become adult in the spring, in April, May or even June. In 1935 they emerged in May, and at Madingley a high degree of parasitism resulted both on larvae and pupae of *Euribia jaceana* and *Eurytoma curta*. There is evidence that though all these hosts are suitable, larvae and puparia of *Euribia jaceana* are preferred. To pool the data from different hosts which are not equally acceptable is not justified if we wish to compare the results with what would be expected if the eggs were distributed at random. Here the distribution is given either for host larvae or, as in the third frequency dis-

tribution in Table 5, for host puparia only. The following corrections have been applied to the 0-groups to allow for the fact that, in some cases where parasites were present, no eggs were found. In the first frequency distribution 348 available host larvae were seen, and parasite eggs were found on 206 of them; but in twenty-three out of the 142 cases in which no eggs were found, a parasite larva was present, or, as in four cases, an adult had already emerged. The 0-group was therefore adjusted to $(142-23)\times 206/229=107$. Similarly, in the second frequency distribution the total number of available larvae was 802, but only in ninety-eight out of 121 which were parasitized were any eggs found, so that group 0 was made equal to $(802-121)\times 98/121=552$. In the third frequency distribution 72/125 of the puparia of *E. jaceana* observed were parasitized, but in five of these no eggs were found; the 0-group was therefore adjusted to equal $(125-72)\times 67/72=49$.

Table 5. *Egg distribution of* Habrocytus trypetae

(1) Sample from Cambridge, observed February 1935: 369 eggs laid in the previous summer distributed amongst 313 host larvae.

(2) Sample from Madingley, observed October 1935: 106 eggs distributed amongst 650 host larvae.

(3) Sample from Madingley, observed June 1935: 187 eggs laid in the spring distributed amongst 116 host puparia. The second calculated frequency is for 187 eggs distributed amongst eighty hosts.

	No. of eggs on the host								
	0	1	2	3	4	5	6	7	8
(1) Observed frequency of hosts	107	104	66	22	7	5	1	0	1
Calculated frequency	96	113	67	26	8	2	0·4	0·1	0
(2) Observed frequency of hosts	552	90	8	0	0	0	0	0	0
Calculated frequency	553	89·4	7·2	0·4	0	0	0	0	0
(3) Observed frequency of hosts	49	13	24	12	8	4	4	2	0
Calculated frequencies:									
(1)	23·1	37·2	29·9	16·1	6·5	2·1	0·6	0·1	0
(2)	7·7	18·1	21·2	16·5	9·7	4·5	1·8	0·6	0·2

When these frequency distributions are compared with those calculated on the assumption that the eggs were distributed singly and at random amongst the hosts, we see that the agreement is very close for the first two frequency distributions, but there is a great excess of superparasitism in the third, in which twice as many hosts escaped parasitism as would have been expected. The application of the χ^2 test to these data indicates that while the goodness of fit is satisfactory in the first two cases ($\chi^2=0{\cdot}026$, $n=2$, $P=0{\cdot}98$; $\chi^2=2{\cdot}0$, $n=3$, $P=0{\cdot}6$), the difference in the last case is strongly significant ($\chi^2=47$, $n=3$, P less than 0·01).

The excessive superparasitism in the third distribution may be due to various factors. The parasite may lay more than one egg on encountering a host. This probably provides the true explanation of the situation, but there is another possibility which cannot be neglected without examination. If the

hosts were not all equally available to the parasites it would be as though the eggs had been distributed amongst a smaller number of hosts. If, for instance, we assume that the total number of available hosts was only eighty instead of 116 we find that the calculated frequency distribution fits groups 1–8 with considerable accuracy (see Table 5), and the difference is not significant; but group 0 is 7·7, where forty-nine were estimated to be present. There is little chance that group 0 should in fact be as low as this, as fifty-three live puparia were examined and found to be without any eggs of *Habrocytus*. As these were in flower heads which were on the standing stems of knapweed (those in the fallen flower heads were not considered in this frequency distribution), they must all in fact have been equally accessible to the searching parasites. We are left with the conclusion that females of this generation often laid more than one egg on encountering a suitable host. This is in accordance with the appearance of the hosts. The eggs of the parasite were in various places in relation to the hosts, being placed either at the top of the gall cell above the larva or puparium, or at the side, or inside a puparium. If eggs were laid by different females on the same host, they would be expected to be placed often on different parts of the host. This was sometimes the case, but the eggs were often to be found in groups or clusters, so that it was frequently difficult to separate the egg shells to count them.

(iii) Torymus cyanimus

This species attacks the fully grown larvae of *Euribia jaceana* in August and September. In the warm summer of 1935 the attack began in August, and some adults had emerged by early September, but most of the larvae apparently spent the winter in the galls. In 1936, which was very much cooler, the attack was not evident till the end of August, and there was no emergence of adults before the winter. In Table 6 the frequency distributions obtained in the summers of 1935 and 1936 at Madingley are given, and also some data for the previous summer taken from a census of knapweed which was grown at the Entomological Field Station, Cambridge. In each case the frequency distribution only includes data from eggs deposited on larvae of *E. jaceana*, and group 0 was adjusted to accommodate for the fact that in certain cases no parasite eggs were discovered when a larva was or had been present. The corrections in the three cases were:

(1) 1532 hosts observed of which 108 were parasitized. No eggs found in nineteen cases. Group $0 = (1532 - 108) \times 89/108 = 1171$.

(2) 321 hosts observed, of which thirty-seven were parasitized. No eggs found in a single case. Group $0 = (321 - 37) \times 36/37 = 276$.

(3) 348 hosts observed, of which fifty-one were parasitized. No eggs found in eleven cases. Group $0 = (348 - 51) \times 40/51 = 233$.

The calculated values in Table 6, which show the frequencies expected if the eggs were distributed at random, are not at all in agreement with the observed values. Groups 0 and 1 are all much smaller than would have been

Table 6. *Egg distribution of* Torymus cyanimus

(1) 185 eggs distributed amongst 1260 hosts at Madingley 1935.
(2) 108 eggs distributed amongst 312 hosts at Madingley 1936.
(3) 204 eggs distributed amongst 273 hosts at Cambridge 1934.

		No. of eggs on the host														
		0	1	2	3	4	5	6	7	8	9	12	13	15	18	38	54
(1)	Observed frequency	1171	47	20	11	5	2	2	0	1	0	0	0	1	0	0	0
	Calculated frequency	1088	160	12	0·6	0	0	0	0	0	0	0	0	0	0	0	0
(2)	Observed frequency	276	15	8	3	2	2	2	1	0	2	0	1	0	0	0	0
	Calculated frequency	221	76·5	13·2	1·5	0·1	0	0	0	0	0	0	0	0	0	0	0
(3)	Observed frequency	233	21	8	1	2	1	0	0	1	1	2	0	0	1	1	1
	Calculated frequency	129	97	36	9	2	0·3	0	0	0	0	0	0	0	0	0	0

expected, and superparasitism is far commoner than if the distribution were random. The third frequency distribution is remarkable in that exceptionally large numbers of parasite eggs were found on single hosts. One flower head contained two hosts, both of which were parasitized; one of them had fifty-four eggs of *Torymus cyanimus* upon it. Most of the eggs had failed to hatch, and one single undersized larva was present, which eventually became adult. The other host had only a single egg upon it. The next flower head examined contained six hosts, with 38, 18, 12, 9, 5, and 1 egg respectively. These two flower heads contained more than half of the eggs found amongst a total of 2174 flower heads, of which 152 contained hosts! If the 204 eggs had been distributed at random 53% of the hosts would have been parasitized; the actual figure was 15%. The flower heads in which the eggs were found were taken from four rows of plants cultivated in Cambridge, and the plants were all close to each other, and all the flower heads appeared to be equally available to the parasites. The probable explanation of the high degree of superparasitism is that the females commonly lay many eggs on a single host. This behaviour, whatever its cause, is extremely wasteful, and contributes largely to the mortality of the species, since one host can at most support a single parasite larva to maturity.

(iv) Eupelmella vesicularis

This parasite has a variety of hosts in the knapweed, and was found to have parasitized some species, such as *Phanacis centaureae* (Kalt.), which live in the stems of knapweed, as well as species such as *Euribia quadrifasciata* Mg. and *E. jaceana* in the flower heads. Most information is available about the distribution of eggs on the puparia of *E. jaceana* which were found parasitized in May, June and early July 1935. In the 6 sq. m. collected in this period there were found sixty host puparia which were available to the parasites, and Table 7 shows how the fifty-four eggs were distributed amongst them. Once again we see that superparasitism is much commoner than would be expected if the egg distribution were random. The eggs were in fact usually laid in small groups, each egg under a separate web of silky material.

Table 7. *The egg distribution of* Eupelmella vesicularis *among sixty puparia of* Euribia jaceana

	No. of eggs on the host							
	0	1	2	3	4	5	6	7
Observed frequency of hosts	40	5	7	3	1	3	0	1
Calculated frequency	24	22	10	3	0·7	0·1	0	0

IV. Discussion and conclusions

(i) *The egg distributions and superparasitism*

Of the parasites studied here, *Eurytoma curta*, the only endoparasite, alone avoids superparasitism. Though superparasitism could perhaps be minimized by systematic search by a single parasite, it can only be avoided by a parasite population by some active process dependent on the recognition of parasitized hosts. Salt (1937, pp. 67–8) has shown that *Trichogramma* recognizes a parasitized host as soon as the ovipositor pierces the chorion, and it has been shown here that *Eurytoma curta* must have a similar sense located in the ovipositor. Such a sense could not easily be used by parasites laying eggs outside the host, and it is noteworthy that so far there are no records of ectoparasites avoiding superparasitism. On the other hand, the following endoparasites are known to avoid superparasitism to a greater or lesser extent: *Trichogramma evanescens* Westw. (Chalcididae), *Ibalia leucospoides* Hochenw. (Cynipidae), *Limnerium validum* Cress. and *Collyria calcitrator* Grav. (Ichneumonidae) (Salt, 1934); *Ooencyrtus kuvanae* How. (Chalcididae) (Lloyd, 1938); *Diadromus collaris* Grav., *Angitia cerophaga* Grav. and *Apanteles plutellae* Kurdj. (Ichneumonidae) (Lloyd, 1940).

Excessive superparasitism can arise in various ways: (1) the eggs may not be laid singly; (2) the hosts may not all be equally available; (3) the search for hosts may not be evenly distributed in space. Besides, there must be little or no tendency to avoid oviposition in parasitized hosts.

There is one example of an endoparasite in which superparasitism is excessive. *Eulimeria crassifemur* Thoms. (a close relative of *Angitia* and *Limnerium*) behaves in this way when attacking two unrelated host species according to Paillot (1923) and Thompson (1939). Besides the ectoparasites recorded here, the Tachinid fly *Centeter*, which lays its eggs on the body of its host *Popillia*, superparasitizes slightly more than would be expected were the distribution random, though only one larva normally matures within the host (Clausen *et al.* 1933, p. 7), and Noble (1932) recorded the same thing for *Habrocytus cerealellae* Ashm. No indication is given by these authors as to the cause of the superparasitism. In the four cases studied in this paper, care was taken to exclude the possibility that the excessive superparasitism might be due to some hosts being unavailable, and parasitism was fairly evenly distributed in all cases except *Eurytoma robusta*. Most samples contained no

eggs of this species, and a few samples contained large numbers. Within these samples the egg distribution was approximately random, but when all samples were considered together there was no doubt that superparasitism was excessive. Eggs of *Habrocytus trypetae*, *Torymus cyanimus* and *Eupelmella vesicularis* were fairly evenly distributed in the different square metre plots examined, and the evidence points to the conclusion that excessive superparasitism arose because the eggs were sometimes laid in groups on the host, even though only one larva could come to maturity.

Salt (1936) showed that the effect of superparasitism on populations of *Trichogramma* under laboratory conditions was to increase the number of host eggs which produced neither caterpillars nor parasites, and to increase the proportion of weak and undersized parasites, and the proportion of males. In the present field studies there was no indication of such effects; even though two or more eggs were frequently found on single hosts, one normal parasite usually emerged. Though superparasitism under these conditions contributes to the mortality of the parasite, this is only of consequence if the wasted eggs might have been laid on other hosts. Smith (1939) emphasizes the view that if a parasite is actually the factor controlling the host density, it is the ability to find hosts, and not the egg supply, which is the limiting factor in the rate of increase of the parasite species. It seems probable from certain unpublished data that the ability to find hosts is indeed the factor limiting egg production in *Habrocytus trypetae*. In such a case then superparasitism is of little economic significance, and the parasite would be unable to control the population of the host at a lower density even if superparasitism were avoided.

(ii) *The searching of parasite populations for their hosts*

Two clear exceptions have been found to the dictum of Nicholson & Bailey (1935) that "the searching of animal populations is always random".

The rare parasite *Eurytoma robusta* appears to search for hosts by random movements which do not overlap, so that the distribution of parasitism is very patchy. Nicholson (1933) was not unaware of this possibility, and remarks that at very low parasite population densities "the animals must be scattered in the environment in more or less widely separated groups, for each female forms a centre from which its offspring diffuse".

The common parasite *E. curta* concentrates its attack in areas in which the proportion of host-containing flower heads is high. How far does this fact invalidate the mathematical theories of populations, such as those of Lotka (1925), Volterra (1926), and Nicholson & Bailey (1935) (for complete references see the review of Thompson (1939)), whose basic assumption is that the encounters between parasites and hosts, or predators and prey, are random, and therefore proportional to the product of the population densities of the species? The population densities used in the formulae of these authors are always mean population densities. Local variations are not considered. The theories are concerned simply with the number of encounters, and not with

their distribution in space. Thus the evidence presented here touches a point which is tacitly omitted in the mathematical theories. The evidence showed that *E. curta* concentrated its attack to a small but noticeable extent in areas in which the proportion of host-containing flower heads was high; but those individual flower heads which contained many hosts were not more heavily attacked. The effect is indeed a small one, which at most increases the speed with which parasites find hosts during the early stages of their search, while the percentage of parasitism is low. The theoretical relationship between the fraction of the area searched by parasites and the percentage of parasitism is expressed by Nicholson's competition curve. The presence of this correlation will cause the curve to rise more steeply at first, but the form of the curve will be unchanged.

In the literature there are three cases of what may be this same phenomenon. Smith & Flanders (1931) and Parsons & Ullyett (1936) noted that local concentrations of host eggs suffer a higher percentage of parasitism by *Trichogramma*. Since the full data are not published in either case it is not possible to assess the precise meaning of the observations. However, Smith & Flanders only noticed the effect when the parasites had been in the plot for a sufficient length of time. If the plots were so widely separated so that *Trichogramma* did not readily move from one to another, this effect might arise in time simply as a result of the more rapid rate of increase in an area of high host density. Howard (1897, p. 51) makes a vague statement to the effect that the primary parasites of the moth *Orgyia* "naturally congregate at the points of greatest host abundance. At points where the caterpillars are scarcer they are thus less exposed to the attacks of their primary parasites". Walker (1940) interprets this, perhaps rightly, to mean that the percentage of parasitism is high where host density is high, and argues therefore that search is not random. However, her own data, which refer to the percentage of parasitism of *Cephus* by *Collyria*, show no trace of this effect, and she concludes that "the behaviour of *Collyria* within the specific environment of the wheat field has been shown to be a fairly good example of 'random searching' in the sense of Nicholson". However, *Collyria* provides a good example of another common type of deviation from random search. Its environmental range is more restricted than that of its host. Hosts in barley are far less subject to attack than hosts in wheat.

Thompson (1939) claims that any theory based on the assumption that search is random is untenable. This view is not accepted here. The exceptions to purely random search are all small discrepancies in spatial distribution. They show that the theories based on this foundation must be used with caution, but within limited areas the theories are likely to be accurate to a first approximation, unless host or parasite population densities are very low, or very uneven. It must be remembered that in many branches of science simple mathematical formulae are used which are known to be inaccurate. One only needs to mention the "gas laws".

It is hoped that in a later paper it will be possible to show that the theory of Nicholson & Bailey provides a reasonable interpretation of the interaction between the various species forming the community in the knapweed flower heads.

V. SUMMARY

1. The egg distributions of five chalcid parasites of the knapweed gall-fly have been studied in a small area.

2. A distinction is made between search for hosts by random movements, and random search in the sense of Nicholson; these terms are defined. Random movements may give rise to a distribution of parasitism different from that expected if search were purely random.

3. The species discussed do not all distribute their eggs at random amongst the hosts. Some species superparasitize the hosts more, and one (*Eurytoma curta*) much less than would be expected if the egg distribution were purely random, owing to peculiarities in oviposition behaviour.

4. Parasitism by *E. curta* is unevenly distributed in space, being higher in areas of high host density. This suggests that search is not exactly random, but is also concentrated in space. A tentative explanation is advanced based on the assumption that search is by random movements.

5. Parasitism by *E. robusta* is very patchy. This non-random distribution is attributed to random movements performed by a very few parasites.

6. It is concluded that these spatial discrepancies do not seriously affect the numerical accuracy of the assumption that search is random in small areas, and that the theory of Nicholson & Bailey may be accurate to a first approximation.

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THE SENSORY PHYSIOLOGY OF THE HUMAN LOUSE *PEDICULUS HUMANUS CORPORIS* DE GEER (ANOPLURA)

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(With 35 Figures in the Text)

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Introduction

Descriptions of the natural history of *Pediculus* (Hase, 1915, 1931; Nuttall, 1917, 1919; Alessandrini, 1919; Buxton, 1939) include a number of scattered observations and experiments on sense organs and behaviour; but, apart from its responses to temperature, the sensory physiology of this insect has never been systematically investigated. In the case of the pig louse, *Haematopinus*, a rather more detailed study has been made by Weber (1929). Later in this paper some of these earlier observations will be discussed in greater detail, but here we may set out briefly the main conclusions reached.

The louse is sensitive to *temperature*: it will pursue a tube of warm water (Martini, 1918), and placed in a temperature gradient it spends most of the time in the region between 25 and 33° C. with a peak at about 29° C. (Martini, 1918; Homp, 1938). *Haematopinus* shows a similar preferred temperature of 28·6° C. (Weber, 1929). Some authors have claimed that the louse has no sense of *smell* (Nuttall, 1917). But *Pediculus* is attracted to the finger even at an air temperature of 36° C. (Martini, 1918) and to cotton-wool impregnated with sweat from the axilla (Pick, 1926); and Weber (1929) showed that *Haematopinus* is repelled by the smell of cedar-wood oil, that it will respond to the finger in preference to an equally warm glass rod and will chose a pig before a dog or a warm oven. There is no experimental evidence in regard to *humidity*, but Nuttall (1917) ascribes the tendency for lice to leave their host in the summer, or during fever, at least in part to the greater humidity of the climate beneath the clothing. *Haematopinus* settles into a state of sleep or akinesis much more readily on a rough surface—an example of thigmotaxis subserved by a sense of *contact*; it is aroused from this state by the vibrations of a tuning fork (Weber, 1929). A definite preference for rough materials is shown by the egg-laying female of *Pediculus* (Hase, 1915; Nuttall, 1917). Both *Pediculus* and *Haematopinus* (Weber, 1929) show an apparent geotaxis; they tend always to climb upwards. But this is probably due merely to the mechanical action of the abdomen (Weber, 1929). As regards *vision*, most authors are agreed that *Pediculus* (Hase, 1915; Bacot, 1917; Nuttall, 1917, 1919) and *Haematopinus* (Weber, 1929) are photonegative and move away from a source of light—though it has sometimes been claimed that this reaction is reversed in the hungry louse (Hase, 1915). *Haematopinus* in a state of akinesis is aroused if exposed to alternate light and shade; and when this louse is moving it may be arrested (akinesis) by sudden exposure to a bright light (Weber, 1929).

What little is known about the sense organs and the mechanism of orientation will be discussed later in this paper.

General methods

The body louse lives normally in the dark on the more or less rough inner layers of the clothing in contact with the warm skin. Under these conditions it doubtless spends the greater part of its life at rest. But in order to show

orientating responses the louse must be moving and it must be visible. The experimental conditions are to that extent abnormal; but there can be little doubt that responses obtained under these abnormal conditions will occur equally in nature.

The method of experiment has consisted in placing the louse in a small circular arena so arranged that one half differs from the other half in a single factor at a time. In most of the experiments the insects have been observed singly: their tracks have been copied on a sheet of paper alongside the apparatus, the position of the louse being marked at 30 sec. intervals. From such tracks it is possible to compare the relative lengths of time spent in either half and to determine whether this is due to the insect (i) coming to rest or moving more

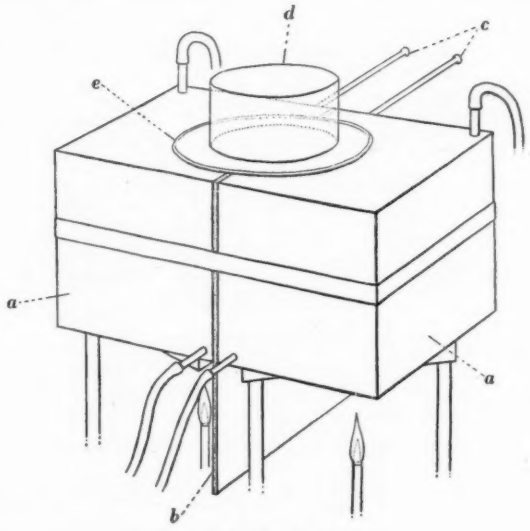

Fig. 1. Explanation in text.

slowly on one side, (ii) changing direction more frequently, or (iii) consistently avoiding one side.

This method has been employed for studying the responses to humidity in *Porcellio* (Gunn, 1937), *Locusta* (Kennedy, 1937), *Blatta* (Gunn & Cosway, 1938), *Culex* (Thomson, 1938) and *Tenebrio* (Pielou & Gunn, 1940) and in studying temperature responses in *Culex* (Thomson, 1938).

The apparatus used is shown in Fig. 1. It consists of two cubical metal containers (*a*) with a side of 15 cm. separated by an asbestos sheet (*b*) 2 mm. thick. Warm or cold water can be passed through the containers or they can be heated from below. Thermometers (*c*) record the temperature of the water immediately below the roof. The arena (*d*) has glass walls and is usually 9 cm. in diameter. The floor is usually of voile stretched on a metal ring (*e*). Except

in the experiments on temperature responses, the arena rests upon one of the containers only, and this is heated to give a temperature on the floor of the arena of about 30° C. Except in the experiments on light responses, the apparatus is exposed to diffuse daylight. Further details will be given as each factor in the environment is dealt with in turn.

Behaviour of the louse in a uniform arena

Most of the experiments have been made on well-nourished adult body lice of both sexes taken directly from breeding capsules worn next to the skin (Buxton, 1939). Hungry lice are sluggish in their movements and sometimes interrupt their course to probe the warm floor of the arena. If females which have not recently had an opportunity of laying eggs are used, they frequently attempt to insinuate themselves between the wall and floor of the arena. Occasionally insects, either fed or unfed, will soon come to rest; but most of the

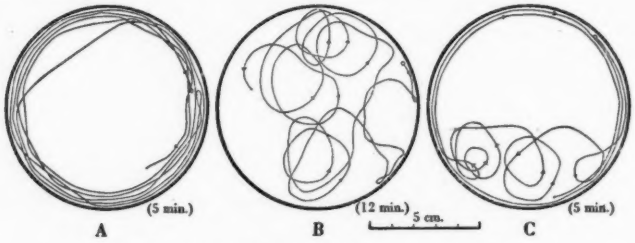

Fig. 2. Tracks followed by lice in a uniform arena.

lice used have continued to crawl without interruption for half an hour or more. Even at the same temperature of 30° C. and with the same voile floor, the rate of movement of different individuals may vary from about 6 to 30 cm. per minute.

The interpretation of the results depends on a knowledge of the behaviour of the louse in a uniform arena. Fig. 2 shows examples of the tracks obtained.

(i) Fig. 2A is the usual type. The louse walks more or less in a straight line; it collides repeatedly with the glass walls, so that the track makes a series of chords in the circle. These chords are usually so short that the track is practically parallel with the circumference;[1] occasionally they cut across the middle of the arena.

(ii) A few insects show a bias towards one side or the other. These follow a spiral course making a series of circles in one direction (Fig. 2B). In some this behaviour is only temporary; but in others it may persist for several days or perhaps permanently. The diameter of the circles varies with the strength of the bias.

(iii) If an insect with a weak left handed bias, for example, moves in a clockwise direction round the arena, it will be prevented by the wall from

[1] In copying these tracks they are shown for the sake of clearness as concentric lines.

turning to the left and will then follow a course around the circumference (Fig. 2C).

REACTIONS TO TEMPERATURE

Method

The two containers (Fig. 1) are brought to the required temperatures and the voile floor of the arena, stretched on a wire ring, placed half on one and half on the other. The arena is open above; its glass walls are 4·5 cm. high; the diameter 9 cm. It rests so that the division between the two containers lies in one diameter. The temperature of the floor is measured by means of a thermocouple resting lightly on the voile.

In most experiments the temperature on one side has been kept at that of the normal environment between the clothes and the skin, 29–30° C.; and the response to temperatures above and below this studied.

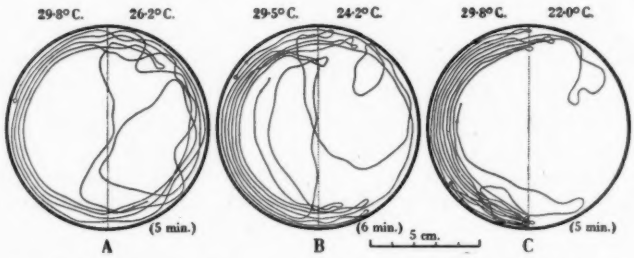

Fig. 3. Tracks followed by a single louse as the temperature in one half of the arena was progressively lowered.

Reactions to alternative temperatures

Some individuals may be indifferent to temperatures as high as 39° C. or as low as 20° C. But these are exceptional. Most show well-marked responses to the change from 30 to 34° C. or from 30 to 25° C.

Fig. 3 shows the reactions of a single louse as the temperature in one half of the arena was progressively lowered. In each case it followed a straight course on the warm side. On passing into 26·2° C. (Fig. 3A) its track was more convoluted and it often turned towards the middle of the arena. On passing into 24·2° C. (Fig. 3B) the reaction was more pronounced and it generally turned back into the warm side after going a short distance. When exposed to 22·0° C. (Fig. 3C) it scarcely crossed the boundary.

Fig. 4 shows some typical reactions of different individuals to rising temperature. Fig. 4A shows the response to 31·8° C. This was the lowest temperature at which an undoubted response was obtained; most individuals were indifferent to this change. Like that to a slight fall in temperature (Fig. 3A) the response shows itself in the tendency of the insect to turn into the arena and to pursue a slightly more convoluted track. Fig. 4B shows a fairly

common type of response to a higher temperature, 35·0° C. The louse follows a straight course at 30° C.; an exceedingly convoluted course at 35° C. Such an insect will spend a far longer time on the adverse side. Fig. 4C shows the usual response to temperatures of 35° C. or higher; the louse turns back instantly on crossing to the warm side. Even where the response fails to cause turning back it may be apparent in the jerky hesitating movements of the insect on entering the adverse side and in occasional convolutions in the trail.

The louse often responds best at the outset and later becomes indifferent (we shall find the same phenomenon in responses to humidity, contact and light). This was well shown in early experiments in which six lice were put together into the arena and with the aid of a stop watch a continuous record made during 10 min. of the number of lice on each side. Offered a choice of 29 and 21° C., out of a total of sixty "louse-minutes" 56·2 were spent at 29° C.,

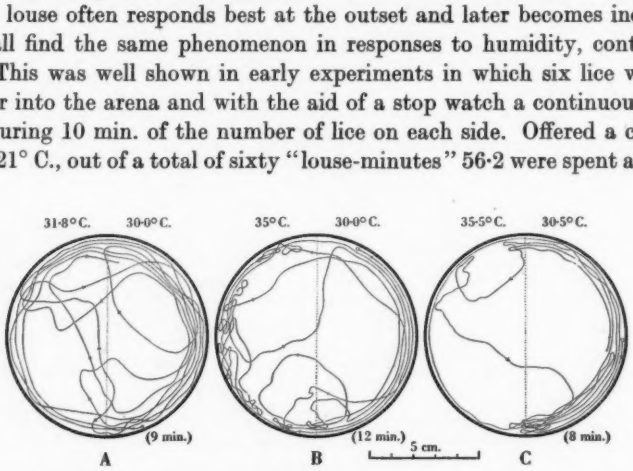

Fig. 4. Tracks followed by different lice when the temperature in one half of the arena was above the optimum.

3·8 at 21° C. On repeating the experiment these figures were 48·5 and 11·5 respectively; and on repeating again 44·9 and 15·1.

The rate of movement is, of course, always greater on the warmer side. For example one insect which appeared indifferent to the temperature change of 31–39° C. moved at an average speed of 27·2 cm. per min. on the cool side, 37·5 cm. per min. on the warm. Under these circumstances the insect of course spends a longer time on the cooler side. Thus one insect which walked continuously round the chamber for 360 sec., spent 220 sec. at 30·5° C., 140 sec. at 39° C.

Once the louse has come to rest it requires a rather high temperature before it is aroused. Ten lice were allowed to settle down into akinesis in a chamber covered with glass. The temperature was then raised slowly during about 20 min. from 32 to 42° C. The lice were aroused at the following temperatures: 39·5, 39·5, 40·0, 40·0, 40·5, 40·5, 41·0, 41·0, 41·0. They do not seem to be aroused by a fall of temperature, at least to 18° C.

An attempt was made to see whether the previous exposure of the louse to a given temperature would influence its response to that temperature in the apparatus. A number of lice were kept at 32 and 27° C. for 1–3 hr. and were

then offered a choice of these two temperatures. No good evidence of adaptation could be obtained. Most insects showed a definite preference for 32° C. (Fig. 5A), irrespective of the temperature to which they had been exposed. But some showed a distinct preference for the intermediate zone, turning back towards the mid-line after passing into 27 or 32° C. (Fig. 5B).

The same experiment was made with insects which had been preconditioned at 35–36 and 25–26° C. and then offered a choice of these temperatures. There was again no evidence that the preconditioning had any influence.[1] The avoidance of 36° C. was on the whole greater than that of 25° C. but the lice often turned back in both directions and several insects crawled along the mid-line swinging alternately towards the left and the right (Fig. 5C). This response to the intermediate zone was more pronounced when extreme temperatures such as 39 and 21·5° C. were present on the two sides (Fig. 5D).

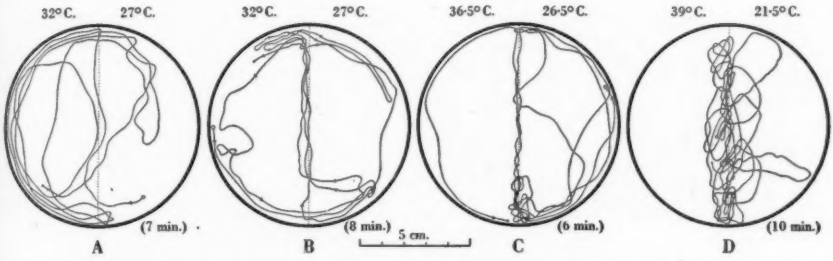

Fig. 5. Tracks of lice when the temperature was above the optimum in one half of the arena and below the optimum in the other half.

Reactions in a temperature gradient

Previous experiments on the response of the louse to temperature have all consisted in observing it in a temperature gradient; sometimes a linear gradient cold at one end and hot at the other (Martini, 1918; Homp, 1938; Weber, 1929); sometimes a concentric gradient around the finger (Hase, 1915) or around a tube of hot water (Homp, 1938). In order to link up the present results with this earlier work the behaviour of lice in a linear gradient has been studied.

The apparatus used is shown in Fig. 6. It consists of an inner trough of sheet zinc (*a*) $45 \times 4 \times 1{\cdot}5$ cm. with a strip of blotting paper along the floor, supported at intervals by metal trestles (*b*) and an outer trough (*c*), also of sheet zinc $50 \times 8 \times 8$ cm. The space between the two troughs is filled with

[1] Homp (1938) describes an attempt to influence the choice of temperature in *Pediculus* by previous exposure for several days at 27–29, 40 and 11–20° C. The results showed no differences at the lower end of the temperature range, no differences in the mean, a very slight (and quite unconvincing) difference at the upper end, where those from 40 and 27–29° C. behave alike, those from 11–20° C. do not extend quite so far.

sand (*d*) saturated with water; into this twelve thermometers are inserted at regular intervals, their bulbs level with the floor of the inner trough. The inner trough is closed above by a series of microscope slides. The outer trough rests on an asbestos strip heated by three small bunsen burners, their flames graded from the warm end. Small blocks of ice (*e*) are placed on the sand at the cool end and the excess water siphoned off by means of a strand of cotton wool (*f*) resting on the sand at the warm end. By this means a pretty constant gradient extending from 13 to 45° C. over the distance of 45 cm. could be obtained. The temperatures registered by the thermometers in the sand agree exactly with those shown by thermometers lying on the floor of the inner trough opposite the corresponding points.

A dozen well-nourished female lice were distributed evenly along this gradient and their positions recorded at the end of 15 min. They were then

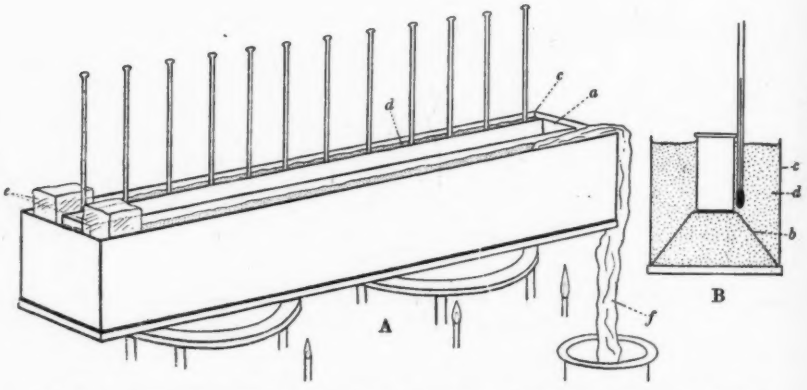

Fig. 6. A, temperature gradient apparatus. B, the same in section. Explanation in text.

removed, evenly distributed again, and the experiment repeated in the same way ten times. At the time of reading, some insects were moving, some at rest. They were observed to come to rest at all temperatures except those above 39° C. (cf. p. 72). Fig. 7 shows the distribution of these lice in relation to the temperature when all the results were summed.

These results confirm those of Martini (1918) in showing the greatest concentration of lice at about 29° C. This has been regarded as the *preferred temperature*. But it is probable that the lice accumulate at this point merely as the result of their avoidance of the upper and lower ranges of temperature. For if individual lice are watched they are seen to crawl for variable distances towards the warm or the cool ends before turning back. Some will turn at 27 or 32° C.; others will continue to 22 or 38° C. Hence at any moment the greater number of insects will tend to be crossing the mean temperature zone of 29–30° C. Moreover, insects approaching from opposite ends of the gradient

tend to huddle together when they meet. This is an additional factor which favours aggregation in the central zone.

Homp (1938) obtained a similar curve for the "preferred temperature". Her curve is based on the relative length of track of individual insects in different segments of the gradient.

Response to radiant heat

Homp (1938) concluded that in the reactions of *Pediculus* to a tube of warm water the air temperature is the important stimulus; experiments to test the effect of radiant heat in the absence of differences in air temperature were unsuccessful. Martini (1918) had concluded that radiant heat, not only from very hot objects but from objects at body temperature, was important

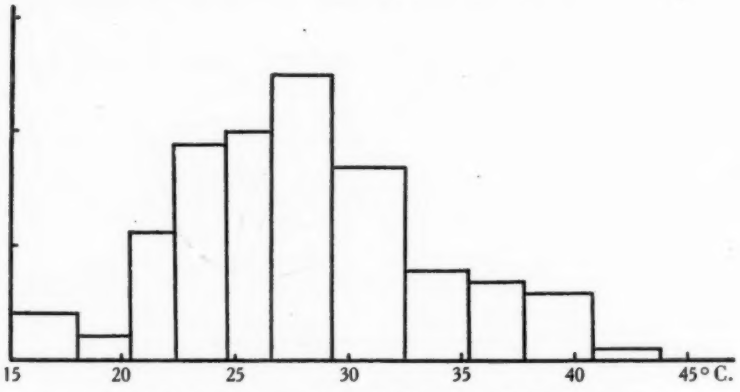

Fig. 7. Relative numbers of lice collecting within different sections of the temperature gradient.

in attracting the louse—but these experiments did not exclude the influence of warm air.

The effect of radiant heat (within the physiological range of temperature) has been reinvestigated as follows. A circular tin 9 cm. in diameter was lined with aluminium foil, and on one half of the wall this was covered with thin cellophane gummed to the surface. The floor of this arena was of blotting paper. It was placed on the bottom of a shallow tin and sealed round the base with plasticine. The whole was firmly secured to the top of the usual container (Fig. 1) through which cold water was passed. Warm water was placed in the tin outside the arena. In this way the walls of the arena were warm while the floor was cooled. In other experiments the outer tin was divided by a partition so that water at different temperatures could be placed in the two halves and thus the wall of the arena heated to different temperatures on the two sides.

The thin cellophane covering makes very little difference to the conduction of heat and consequently to the gradient of air temperature from the walls to the centre of the arena. But it makes a great difference to the radiant heat. The emissivity of the aluminium covered by cellophane is almost equal to that of a dull black surface; the emissivity of the aluminium alone is only about 5 % of this (Crowden (1934) and personal communication).

Results. When the air temperature against the wall of the arena was 29° C. all round and the temperature at the centre of the arena 24·5° C., the lice all made their way to the wall and moved round close to it, showing no difference in the two sides (Fig. 8A). The same result was obtained when air was blown through a funnel above the arena. This reduced the gradient of air temperature, making this 24° C. at the centre, 25° C. against the wall.

When the air temperature against the wall was 43° C. all round and that at the centre 31° C., the lice were repelled and turned away from the wall

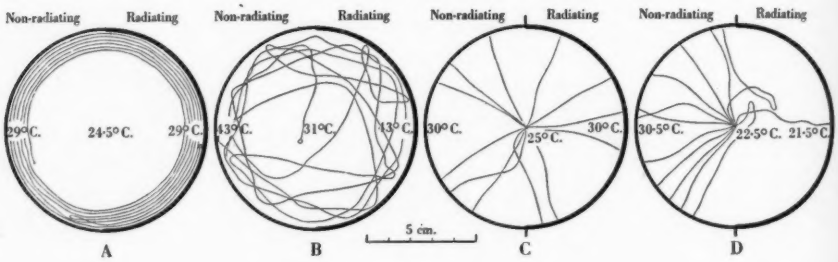

Fig. 8. Reactions of ice to radiant heat.

before actually coming in contact with it. But they came no nearer to the wall when this was covered with aluminium alone (Fig. 8B). In this repellent effect it was clearly the air temperature that was important.

If a number of lice are liberated together in the centre of the arena they crawl over and over one another and then one by one they leave the cluster and migrate to the periphery. When the air against the wall of the arena was 30° C. all round and that at the centre 25° C. the lice moved out in all directions going indifferently to the radiating cellophane surface and the non-radiating aluminium surface (Fig. 8C). Whereas, if the air temperature around the wall is 30·5° C. against the aluminium and 21·5° C. against the cellophane they almost all go towards the former (Fig. 8D).

It is clear that over the range of temperatures used in these experiments, which is that encountered by the louse in nature, in comparison with warm air radiant heat is of no importance in orientation (cf. Wigglesworth & Gillett, 1934*b*).

REACTIONS TO HUMIDITY

Method

Fig. 9 shows the arrangement of the arena for experiments on atmospheric humidity. A metal base plate (*a*) 13 cm. square rests on the top of the warm tank. A rod (*b*) 1·5 mm. in diameter is soldered to the base plate. On either side of this is placed a semicircular pad (*c*) made up of four thicknesses of blotting paper, which will hold 5 c.c. of the solution for controlling the humidity. A disk of perforated zinc (*d*) is separated from the pads by four very thin wire rings. The voile floor (*e*) stretched on a wire ring rests on the perforated disk. The arena is provided by the lid of a Petri dish (*f*) 9 cm. in diameter, ground down so as to measure 5 mm. deep inside. A glass rod (*g*) is fastened with plasticine across the roof of the arena so as to reduce the area of contact between one half of the chamber and the other. A large Petri dish (*h*) 11 cm. in diameter covers the entire apparatus so as to prevent evaporation. With very high humidities, dew may form on the inner cover (*f*); this is prevented by *slightly* warming the outer cover (*h*) before each experiment.

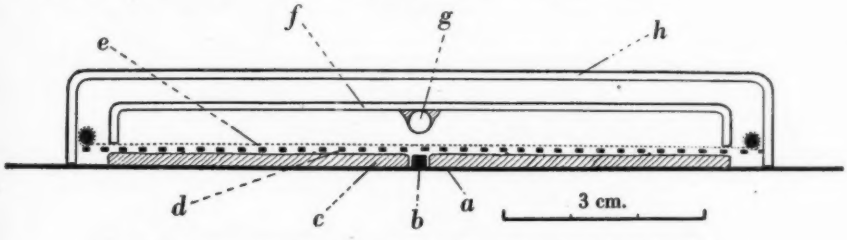

Fig. 9. Arrangement of the arena for experiments on atmospheric humidity, seen in cross-section. Explanation in text.

The humidity has been controlled by the use of saturated salt solutions (Buxton, 1931; Buxton & Mellanby, 1934), 5 c.c. being added to a fresh pad each day. The following salts were used, all at 30° C.: $Ca(H_2PO_4)_2$, 95 %; neutral Na-tartrate, 92 %; KCl, 85 %; NaCl, 76 %; NH_4NO_3, 60 %; $Ca(NO_3)_2$, 47 %; $MgCl_2$, 32 %; K-acetate, 16 %; $ZnCl_2$, 10 %. It is assumed that the humidity on the surface of the voile is that given theoretically by the salt mixture which is about 1 mm. below. It may be noted that the area of contact between each half of the chamber and the salt solution is 31·8 sq. cm.; whereas the area of contact between the one half of the chamber and the other is 1·8 sq. cm. Hence the gradient of humidity in the mid-line is probably very steep. This is borne out, as will be seen, by the behaviour of the insect.

Reactions to alternative humidities

Before using controlled humidities with this apparatus some preliminary experiments were made in which a sheet of blotting paper was divided by a line 1–2 mm. wide impregnated with paraffin wax, and one half left dry while

the other was saturated with water. The arena was open above and consisted of a ring of glass prepared from a Petri dish by grinding away the floor.

Fig. 10A shows a typical result. The louse remained the whole time on the dry side; it often turned away while still a centimetre distant from the moist surface. This response was not due to a difference in temperature. For the temperature as measured by a small thermometer with the bulb resting on the surface was 30·0° C. on the dry side, 30·5° C. on the moist—the improved conduction of heat through the moist paper more than compensating for the cooling effect of evaporation.

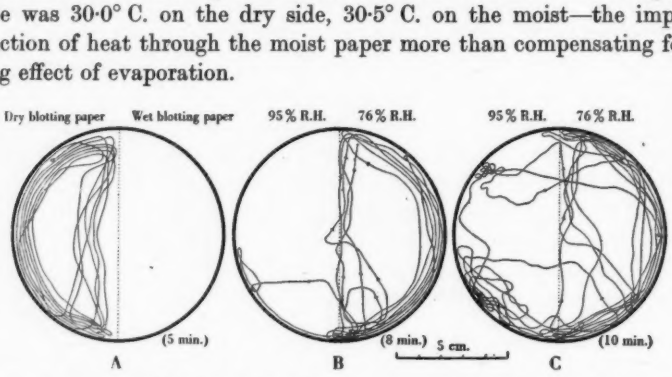

Fig. 10. A, response of louse to a wet surface. B, C, responses to atmospheric humidity.

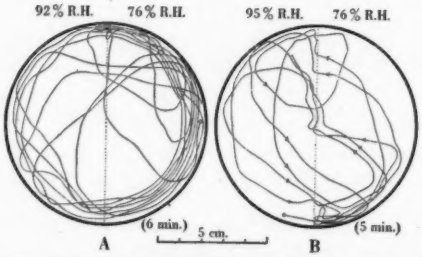

Fig. 11. A, track followed by louse showing a very weak response to high humidity. B, track of louse with left hand bias which showed a fairly strong reaction to humidity.

Fig. 10B, C, shows the response of lice, taken directly from the breeding capsules, to a choice of atmospheric humidities of 95 and 76 %.[1] There is a very striking avoidance of the higher humidity. Sometimes (Fig. 10B) the insect turns back immediately on reaching the moist side. If it fails to do so (Fig. 10C) it frequently pursues a very convoluted course before regaining the drier side. During the periods on the moist side its movements are very agitated.

This reaction to high humidity varies in intensity; sometimes it is absent and the insect is indifferent. Fig. 11A gives an example of a very weak response to 92 % R.H. in the presence of 76 % R.H. It is apparent only in the

[1] The temperature, as measured by a small thermometer within the closed chamber, was 30·25° C. on each side.

slightly more convoluted course on the moist side. Fig. 11B is an example of an insect with a weak left hand bias. It regularly turns away on coming to the moist side; but as the result of the left hand bias these turns fail to take it back into the drier side.

When experiments were made at other parts of the humidity range curious inconsistencies became apparent. Many insects would show a very definite reaction to a given pair of alternatives, others would be indifferent under the same conditions, while a few would show an equally definite reaction in the opposite sense. This suggested that the response might be influenced by the conditions to which the insect had been previously exposed.

This was tested as follows. Before each experiment the lice were divided into two groups, usually six or ten in each. Half were exposed for 1–3 hr. to one of the humidities to be used and half to the other. Most of the experiments consisted in offering a choice of 95 % R.H. and a series of lower humidities, or of 10 % R.H. and a series of higher humidities. The following are summaries of the results:

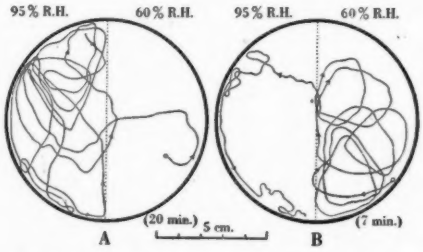

Fig. 12. A, track of louse which had been kept at 95 % R.H. for 2 hr. B, track of same louse after exposure to 60 % R.H. for 10 min.

(i) 95 %/85 %. Insects accustomed to 95 % R.H. show some avoidances of 85 % R.H., but most gradually come to prefer 85 % R.H. and avoid 95 % R.H. Most of those from 85 % R.H. show consistent avoidance of 95 % R.H.

(ii) 95 %/76 %. Accustomed to 95 % R.H. they show at the outset definite avoidances of 76 % R.H.; but later the response is reversed and they avoid 95 % R.H. Those from 76 % R.H. show consistent avoidance of 95 % R.H. or are indifferent.

(iii) 95 %/60 %. Those from 95 % R.H. may avoid 60 %; but this reaction is easily reversed. Fig. 12 gives an example of this. Insects from 60 % R.H. consistently avoid 95 %.

(iv) 95 %/47 %. After exposure to 95 % R.H. the lice usually show at first consistent avoidance of 47 % R.H., though later they may become indifferent. Those from 47 % R.H. show variable responses: some consistently avoid 95 % R.H.; others react in this way at first, but later the response is reversed and they avoid 47 % R.H.

(v) 95 %/32 %. The response is again influenced by the humidity to

which the insects had been exposed; but in both groups they tend to avoid 32 % R.H. eventually.

(vi) 95 %/10 %. Insects from 95 % R.H. avoid 10 % R.H. consistently. Those from 10 % R.H. usually show a number of avoidances of 95 % but later the response becomes reversed and they avoid 10 %.

(vii) 10 %/32 %. One insect after exposure to 10 % R.H. gave some definite avoidances of 32 % R.H. Those from 32 % R.H. were indifferent.

(viii) 10 %/60 %. There was usually no evidence of any preference between these two humidities, but those from 10 % R.H. show occasional avoidances of 60 % R.H.

(ix) 10 %/76 %. The responses are rather weak but avoidances of 76 % R.H. occur in both groups.

(x) 10 %/85 %. Insects from 10 % R.H. consistently avoid 85 % R.H. Those from 85 % R.H. show a few reactions against 10 % R.H., but soon come to avoid 85 % R.H.

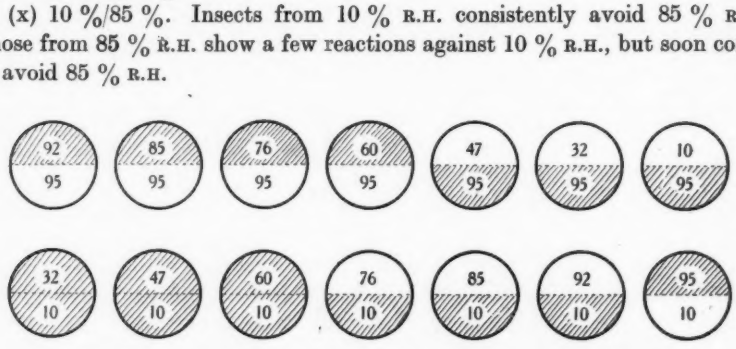

Fig. 13. Diagram summarizing the preferences of the louse when offered two humidities. The shaded side is that usually chosen.

(xi) 10 %/92 %. Lice from 10 % R.H. avoid 92 % continually. Those from 92 % R.H. may be indifferent or react away from 10 % R.H.; but this response is sometimes spontaneously reversed.

(xii) 10 %/95 %. As already described under (vi) insects from both groups come to prefer 95 % R.H. and avoid 10 % R.H.

Thus the response of the louse in the presence of two humidities is greatly influenced by the conditions to which it has previously been exposed. But whatever the conditions it usually comes eventually to prefer the same one of these two humidities.

These results are summarized in Fig. 13. Two points emerge. (i) The louse is usually more or less indifferent to humidity over the drier part of the range—from 10 to 60 % R.H. (ii) The reaction towards a given humidity is influenced by the alternative which it is offered. Thus in the presence of the lower humidities, 10, 32 and 47 % the high humidity of 95 % is preferred; whereas in the presence of the higher humidities, 60, 76 and 85 %, 95 % R.H. is avoided. And whereas 10 % R.H. is preferred in the presence of 85 or 92 % R.H., it is avoided in the presence of 95 % R.H.

The meaning of these differences is not clear. It seems, however, that the insect (*a*) prefers a low or medium humidity, (*b*) avoids any change once it has become accustomed to a given humidity. Now it is a general property of hygroscopic substances that they absorb water more rapidly from a very moist atmosphere than they lose it in a dry. If therefore, as seems probable, the sense organs concerned behave like other hygroscopic materials this might explain why the insect comes to prefer 95 % R.H. before 10 % R.H., but to prefer 10 % R.H. before 85 or 92 % R.H. But it is not easy to explain along these lines the striking avoidance of 95 or 100 % R.H. in the presence of 60, 76 or 85 % R.H.

In general the louse shows a greater sensitivity within the higher ranges of humidity. For example the louse is usually indifferent between 10 and 60 % R.H. but may show a striking preference for 92 % R.H. in the presence of 95 % R.H. This was observed also by Thomson (1938) in the mosquito *Culex* and by Pielou & Gunn (1940) in the adult mealworm *Tenebrio*. As these authors point out, it suggests that the sense organs are reacting like hygroscopes to relative humidity (Pielou, 1940).

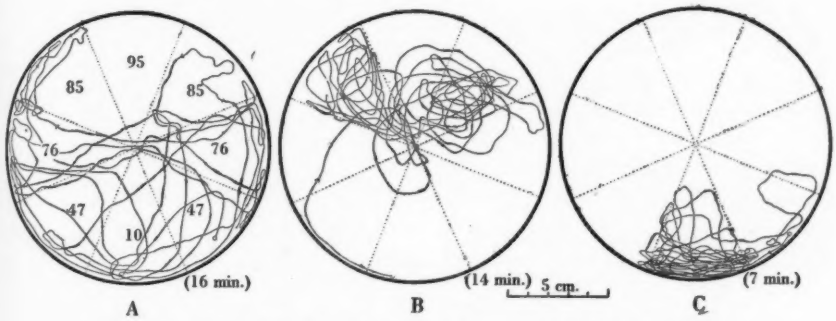

Fig. 14. Tracks of single lice in an arena divided into sectors of different humidity, as indicated in A.

Reactions in a humidity gradient

Some experiments were also made in which the louse was offered a wide range of humidities. A large arena formed by a Petri dish 13·5 cm. in diameter was prepared in the manner already described and divided into eight sections each with four thicknesses of filter paper saturated with salt solution below the perforated zinc. The humidities were arranged as shown in Fig. 14, which gives some typical results on lice taken direct from the breeding capsules.

Fig. 14A represents the most usual response. The insect is apparently indifferent over the range from 10 to 76 % R.H., but avoids 85 % R.H. and still more strongly 95 % R.H.

Fig. 14 B is an example of spontaneous adaptation to a high humidity (85 % R.H.) and avoidance of change in either direction. This result was again

obtained on repeating the experiment with the same insect. A few other similar, though less striking examples were obtained.

Fig. 14C is a very unusual case of adaptation to 10 % R.H. with avoidance of all higher humidities. Later this response was gradually lost and the type shown in Fig. 14A was given.

Lice were also tested in a linear gradient of humidity. The temperature gradient apparatus (p. 74) heated throughout to 30° C. was used. A glass tube of 1 cm. internal diameter (Fig. 15*a*) was ground down so as to form a cover 45 cm. long and inverted over a rectangular zinc framework (*b*) with a floor of bolting silk (*c*). Below this was a series of 9 pads, each 5 cm. long, composed of four thicknesses of blotting paper (*d*) enclosed between two strips of perforated zinc (*e*). The gradient consisted of the following relative humidities: 10, 16, 32, 47, 60, 76, 85, 95, 100 %.

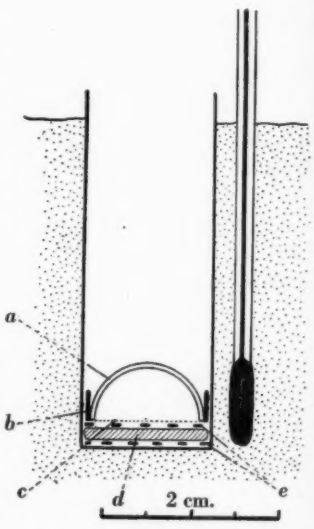

Fig. 15. Section of humidity gradient apparatus. Explanation in text.

A dozen lice were introduced at a time and their positions recorded at 5 min. intervals. In one experiment, after 7 readings in the course of half an hour the summed results were as follows: 10 % R.H. 49; 16 % R.H. 12; 32 % R.H. 6; 47 % R.H. 7; 60 % R.H. 3; 76 % R.H. 4; 85 % R.H. 3; 95 % R.H. 0; 100 % R.H. 0.

The insects obviously avoided 95 % R.H. and in some cases 85 % R.H. They then turned back and continued walking until they reached the dry end. Here they formed a cluster, from which occasional insects would come out, move as far as 85 or 95 % R.H. and then return. The aggregation at 10 % R.H. is thus of no significance. The insects used appeared quite indifferent from 10 to 76 % R.H.

Reactions to smell

Method

For experiments on smell the arena is open above and consists of a ring of glass made from a Petri dish by grinding away the bottom. The rest of the apparatus is the same as that used in the humidity experiments, the blotting-paper pads being replaced by whatever materials are to be tested. The floor of the arena is always at about 30° C. Under these conditions it is assumed that the warm odours will be carried upwards by the convection currents and produce in still air a pretty sharp boundary between the two halves.

Results

A double layer of cotton stockinet was placed below each side, that on one side being clean, that on the other having been pinned inside the shirt and worn next the skin for a week or 10 days. Fig. 16A shows the insect returning to the man-scented side almost immediately after crossing into the neutral side. Fig. 16B shows the track of a louse which followed a straight course on the man-scented side, a convoluted course on the neutral side. Fig. 16C represents a more extreme example of the same type.

The same experiment was repeated using stockinet which had been well rubbed on a dog, together with some of the dog's hair. Most of the lice proved

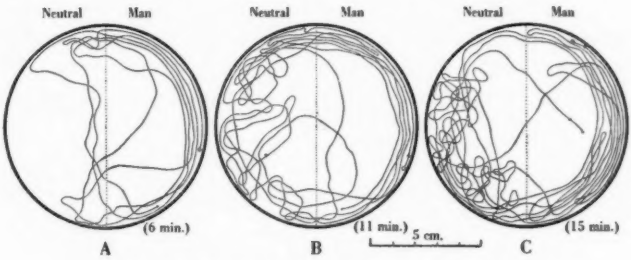

Fig. 16. Reactions of lice to the smell of man.

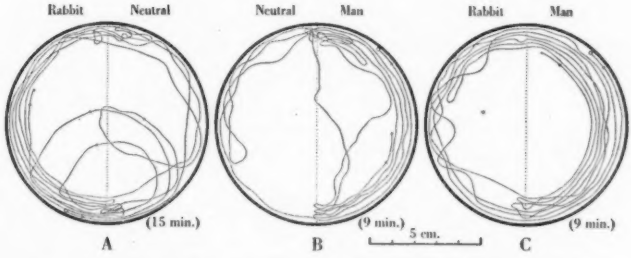

Fig. 17. Reactions of the same louse to the smell of man and rabbit.

indifferent; a few showed a very slight preference for the dog-scented side. When lice were offered a choice of a man-scented side and a dog-scented side they showed a preference for the former quite as great as that shown in Fig. 16A, where one side was neutral.

The response to the odour of rabbit was tested in the same way. (To the human sense this odour comes much closer to that of man.) Most lice show a quite definite preference for the rabbit-scented side when the other side is neutral. The reaction to human scent is, however, stronger. And when the rabbit-scented side is exposed alongside the man-scented side there is a definite preference for the latter. Fig. 17 shows the reactions of the same insect when offered these three alternatives.

An attempt was made to see whether lice would show any detectable preference for the odour of the individual human host on which they had been reared. A series of lice reared throughout on S. A. S. and another series reared on V. B. W. were offered the choice of stockinet worn for several days under the clothing of these two hosts. No evidence was obtained of the one host being more attractive than the other, or of the lice being more strongly attracted by the odour of the host on which they had been reared. But the experiments were not extended to other human hosts.[1]

Other odours which might be expected to contribute to the normal environment of the louse are those from other lice or from their excreta. A Petri dish exactly the same size as the arena was ground down so as to be only 4 mm. deep inside. Clean filter paper was placed on the bottom and it was divided in the middle by a partition of celluloid. Thirty-six female lice were introduced into one side of this container. The voile disk was placed on the top, and on this the open arena. Fig. 18A, B shows two examples of the tracks followed

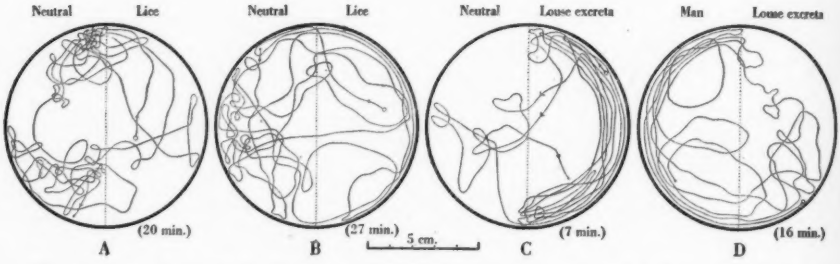

Fig. 18. A, B, tracks of single female lice when half the arena covered a chamber containing other female lice. C, reaction to the smell of louse excreta. D, reaction to smell of man when the other half of the arena contained louse excreta.

by single female lice placed in the arena. They follow a much more convoluted course above the empty side than above the side containing the other female lice. During the experiment the latter laid a number of eggs at various points in the chamber. (Bacot (1917) and Nuttall (1917) have shown that females tend to lay their eggs where they or other females have already deposited them.) The lice in the container also produced a small amount of excreta.

The response to excreta was tested by covering a pad of blotting paper with excreta and cast skins collected from the breeding capsules, moistening this layer and then allowing it to dry. The material contains much incompletely digested blood; on warming to 30° C. it gives off a slight and somewhat disagreeable smell. It was compared with a clean pad of blotting paper. Fig. 18C shows a very striking response, this insect being almost confined to the side above the excreta. If a piece of man-scented stockinet is placed below the

[1] There are many inconclusive statements in the literature about individuals who are said to be repellent or particularly attractive to lice (Frickhinger, 1916; Alessandrini, 1919; Pick, 1926).

opposite side the lice come to prefer this side and avoid that above the excreta. Fig. 18D shows an example of this.

It was interesting to see whether the attractiveness of human odour was enhanced when the smell of lice and their excreta was added to it. A piece of man-scented stockinet was divided and half placed in each side of the chamber described above. In addition, one side contained thirty-six lice of mixed sexes and a large quantity of excreta. As shown in Fig. 19 the lice in the arena show a slight preference for the side containing the lice and excreta.

In the past many experiments have been made with the practical object of discovering a repellent which will prevent the biting of lice when applied to

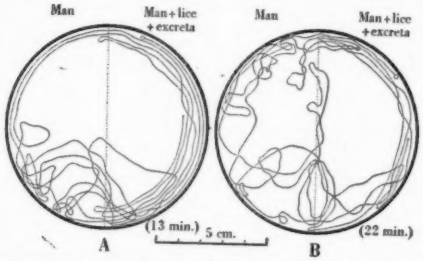

Fig. 19. Tracks of lice where half arena provided smell of man and half that of man plus lice and excreta.

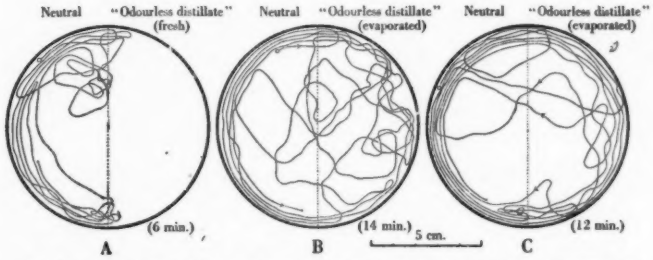

Fig. 20. Reactions of lice to the smell of petroleum ("odourless distillate"). A, fresh; B, C, after exposure for 18 hr.

the skin. These tests have proved consistently negative and have even led some authors (Nuttall, 1917) to question whether lice possess a sense of smell. But a variety of substances, light oils, creosote, naphthalene, etc., will serve as repellents when studied by the present technique. A rather mild repellent chosen for the experiments described was "Shell odourless distillate". This is a highly refined petroleum oil, containing less than 1 % of aromatics, with a boiling point range of 198–257° C. It has a faint smell of petroleum, which disappears from a thin film after exposure to the air for some hours.

A few drops of "odourless distillate" were applied to filter paper below one half of the arena, clean filter paper was placed below the other half. Fig. 20A shows the response of a louse to the fresh oil; this is almost completely

avoided. Figs. 20B, C show responses after the film had been exposed to the air at room temperature for 18 hr. and no longer had a smell detectable by man. The insect now merely follows a more convoluted course on the side above the evaporated oil.

Reactions to contact

Method

Most of the experiments were on the same lines as before. Materials of graded roughness were secured with gum to glass plates. These were placed side by side, warmed to 30° C., and a small Petri dish 6 cm. in diameter inverted over them to form the arena. The materials used were glazed paper, unglazed paper, blotting paper, smooth silk, cotton stockinet and coarse woollen stockinet. The texture of the last three in relation to the louse is shown in Fig. 21.

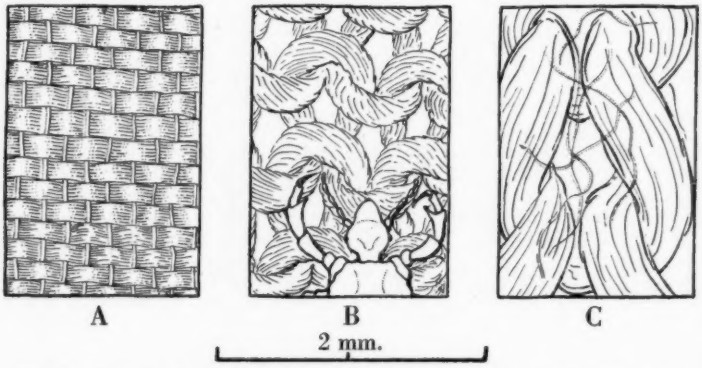

Fig. 21. Texture of materials used in relation with the louse. A, silk; B, cotton stockinet; C, woollen stockinet.

Results

Lice cannot hold to glazed paper. When on any rougher material they will usually avoid glazed paper absolutely. If they do walk onto it they struggle actively, their claws slipping over the surface, until they get back to the rougher material (Fig. 22A).

When offered a choice of other materials they show a varying degree of preference for the rougher. Fig. 22B shows the track of a rather slow-moving insect offered woollen stockinet and smooth unglazed paper; it never left the woollen stuff. Fig. 22C is the track of a louse offered this woollen material and smooth silk. It seldom left the margin of the wool; and when it did so it showed great agitation in its movements, followed a convoluted course, and soon returned. Fig. 22D shows the response to woollen stockinet and fine cotton stockinet. The reaction is not so strong; the louse often gets on to the cotton stuff and there follows a somewhat convoluted course. Fig. 22E is the

trail of an insect in the presence of this same cotton stockinet and smooth silk. It repeatedly gets onto the silk, and then twists and turns in all directions before returning to the cotton. Fig. 22F is a response to the cotton stockinet and blotting paper. On the cotton the louse follows a straight course; on the blotting paper it changes direction repeatedly and pursues a highly convoluted trail.

These figures provide a fair sample of the results obtained. But there is a great deal of individual variation. It is obvious that the course followed upon a given material depends upon what alternative material is offered. If any of the materials is present alone, the trail soon becomes straight. And it is

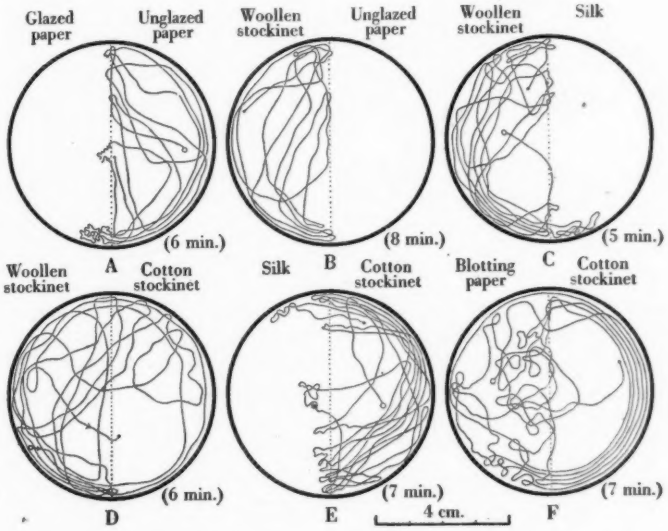

Fig. 22. Reactions of lice to contact with rough and smooth materials.

quite common for the insect to be completely indifferent even when two very dissimilar materials are present; or for it to become indifferent and follow a straight track on both sides in the course of an experiment.

The general response of turning back from a smooth material is seen equally when a narrow strip of khaki flannel is placed on blotting paper (Fig. 23A); or even when a strip of frayed flannel is laid, like a seam in the clothing, upon a floor of the same material (Fig. 23B). On the other hand, the louse shows little tendency to move along in contact with a smooth surface. It often turns and leaves it almost at once (Fig. 23C). And most of the time that it walks round the Petri dish it is not in contact with the walls nor does it touch the wall with its antennae. As described already (p. 70) it follows a series of short chords in the arena.

Some other tactile responses may be mentioned. Lice show a great tendency to creep into narrow clefts of rough material. This is particularly so in the egg-laying female. On the other hand they do not seem inclined to insinuate themselves beneath smooth surfaces, such as a coverslip, whether on smooth or rough material. They tend to come to rest (akinesis) more readily on rough material and scarcely ever on a glazed surface. This has already been noted in *Haematopinus* by Weber (1929).

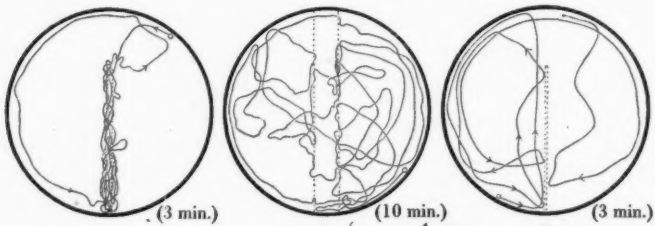

Fig. 23. Tracks followed by lice: A, a strip of flannel on blotting paper; B, a frayed strip of flannel on a flannel surface; C, a vertical strip of metal on blotting paper.

Reactions to moving air

Air currents provide another form of mechanical stimulus which might be important in the orientation of the louse; although the relative shortness of the antennae and the sparseness of slender tactile hairs makes this unlikely. The effect was tested in an arena 9 cm. in diameter and 4·5 cm. deep, open above and with a voile floor. This was divided by a vertical card extending from

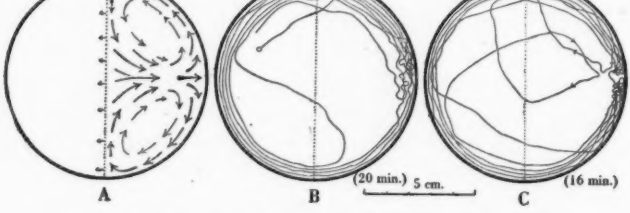

Fig. 24. A, the course of air currents in the arena. B, C, tracks of lice in such an arena.

the top to within 3 mm. of the floor. A jet of compressed air blown through a pipette was directed downwards and outwards at an angle of 45° on one side of the arena, setting up a violent turbulence there. The direction of the currents was mapped out by means of the parachute of a dandelion seed (Fig. 24A). Opposite the jet the current was so strong that the louse was blown violently

away on a smooth surface. Only very weak air currents pass below the partition to the other side. The floor of the arena was at room temperature, about 20° C.

Fig. 24B, C, gives typical results obtained. The louse shows no response until it enters the very strong current close to the jet. It then stops and turns in all directions and frequently retreats.

REACTIONS TO LIGHT

Method

The reactions so far considered (apart from those to radiant heat) have all concerned diffuse stimuli. Such stimuli may show a gradient of intensity; but the insect, at a given point in the gradient, cannot detect, without further exploration, in which direction it is rising or falling. In the case of light, however, it is important to differentiate between the effects of general light intensity and the effects of directed light. As Ullyott (1936) has shown, it is very difficult to arrange an arena with a large difference in illumination on the two halves without at the same time introducing differences in the amount of light reflected from the surroundings.

In observing the track of the insect, however, there is no need to use a divided chamber; because the intensity of light can be changed instantly all over the arena. To test the effects of light intensity an open glass ring with walls 1 cm. high, painted a dull black inside, was used. This was exposed in a darkened room to a screened lamp vertically above the centre and the light intensity varied by interposing a number of pieces of stout white card below the lamp. The intensity of the surface illumination was measured approximately with an "Avo" photoelectric cell (Automatic Coil Winder and Electrical Equipment Co., Ltd.); approximate values for the lowest illuminations lying outside the range of this instrument were arrived at, by extrapolation, from the number of cards interposed.

Other methods will be described below.

Reactions to changes in light intensity

Lice were kept in the dark for an hour or so and then exposed in the arena to a surface illumination of less than 0·01 metre candle. At this illumination it is just possible to follow the trail of the louse. The illumination was then suddenly increased to 500 metre candles.

(i) The most constant response is for the movements of the insect to be arrested by the sudden increase in illumination. It may stop only for a few seconds or it may remain for many minutes in a state of akinesis. This response is described by Weber (1929) in *Haematopinus*. When the louse advances again, spontaneously or after being disturbed by touching, it usually moves at a slower pace and often hesitates or stops. Eventually it becomes accustomed to the light and regains its normal activity. Thus in one experiment the louse

at an illumination of 0·008 metre candle moved round the arena for 5 min. at an average rate of 25·4 cm. per min. On exposing it to 500 metre candles it stopped for 10 sec.; advanced 5 cm. and came to rest again. It was disturbed by touching after 5 min. It went once round the arena at a rate of 18·8 cm. per min. and stopped once more. It was aroused by touching after 5 min., and then continued around the arena at an average of 22·6 cm. per min. during the next 5 min.

(ii) But there is a second type of response in which the movements of the insect are not notably retarded but the course becomes convoluted on exposure to the bright light (Fig. 25). This is the type of response we have seen already in the presence of slightly adverse stimuli of temperature, humidity, smell and contact.

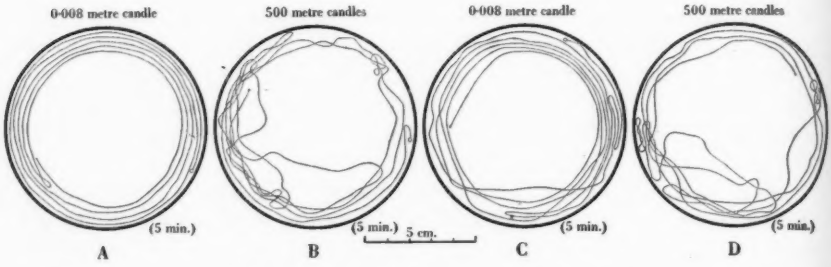

Fig. 25. Consecutive tracks of a single louse when the surface illumination was changed abruptly from 0·008 metre candle in A and C to 500 metre candles in B and D.

Reactions to directed light

It has repeatedly been observed that lice move away from the light (Hase, 1915; Nuttall, 1917; Weber, 1929). If the arena is placed unshaded before the window the tracks of the lice are mostly on the opposite side (Hase, 1915). In this reaction, however, the intensity of the diffuse light to which the louse is exposed will have an important effect. For, as Ullyott (1936) points out, in accordance with the Weber-Fechner law the threshold value of the orientating stimulation should be a function of the total stimulation to which the animal is subjected.

This effect is readily demonstrated by having one half of the arena with the wall dull black, the other half with white paper on the outside of the uncovered glass. It is illuminated from above so as to give a uniform surface illumination of 500 metre candles. A black card is then arranged so as to throw that half of the arena with black walls into deep shade (less than 0·1 metre candle).

Fig. 26 shows the result of such an experiment. When the arena is bright all over (Fig. 26A) the louse walks closely round the black wall, but tends to swing away from the white wall into the middle of the arena. Whereas when

the black-walled side is shaded (Fig. 26A′) the louse may be entirely confined to the dark side, often turning back well before it enters the bright light. But if it does stray into the bright side it follows the same type of course as before—merely swinging away from the white wall (Fig. 26B).

It is evident therefore that the light reaching the louse horizontally from different directions is chiefly important in its orientation. This can also be seen when a relatively small part of the wall of the arena is darkened. In the experiment shown in Fig. 27 the arena was placed in front of the window. It

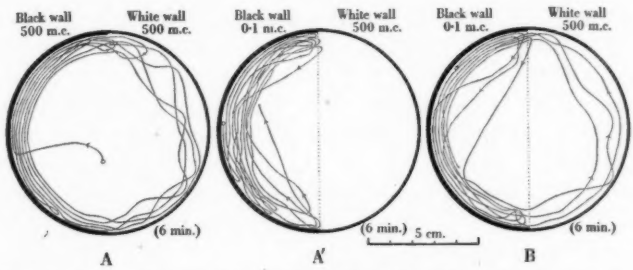

Fig. 26. Reactions of the louse to light reflected from the walls of the arena. A, entire arena light; A′, track of same louse with black-walled half of arena shaded; B, track of another louse under the same conditions.

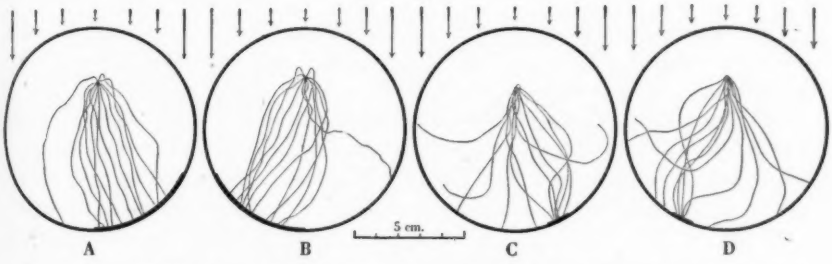

Fig. 27. Tracks of lice liberated in the middle of an arena with a black segment in the otherwise white walls. The arrows show the direction of the light.

was lined with white paper save to one side or the other of the pole opposite the window, where there was a dull black segment 5 cm. wide. The wall was 1·5 cm. high. Twelve lice were liberated in turn at a point in the arena; and as can be seen in Fig. 27A, B, almost all went to the black region as they moved away from the window.

When the black segment was reduced to 1 cm. wide lying 1·5 cm. to one side or the other of the mid-line, the deflection of the tracks towards the black region is still apparent—particularly when they come close to it (Fig. 27C, D). Bacot (1917) has already described how lice will turn towards dark objects and Homp (1938) observed that they will avoid a glass rod set in their way as

they move from the light. (Here they are presumably repelled by the reflected light.)

A response described by Weber (1929) in *Haematopinus* occurs also in *Pediculus*—the louse in a state of akinesis is awakened by alternating the light from bright to dark every few seconds.

According to Hase (1915), starved *Pediculus* become "positively phototropic"—except when disturbed, in which case they are negative as usual. I have been unable to confirm this observation. Twenty lice starved for 18 hr. at 28° C. and then for 24 hr. at 22° C. were placed in the centre of an arena formed by inverting a 13 cm. Petri dish on voile in front of a window, with the half away from the window shaded with black paper. When placed in the arena all the lice moved to the shaded half. Here they wandered for some time and all eventually settled down, mostly in the shade. Left undisturbed for 6 hr. they still remained in the same resting places; none moved toward the light.

Nuttall (1919) showed that lice offered black and white cloth, collect chiefly on the black. Under these conditions both directed light and general light intensity will be operative.

Sense organs

Antennae

Fig. 28A shows the antennae of the adult louse. I can detect no constant differences between the sexes. It consists of five segments which bear three types of sensillum.

(i) *Peg organs.* These form a group arising from the thin cuticle at the apex of the 5th segment. There are nine or ten on each antenna: three sharply pointed, lying dorso-lateral, and six, or usually seven, of varied length, with rounded tips, lying medial and ventral. In section they are seen to be exceedingly thin-walled (Fig. 28D). Below each is an elongated group of about six sense cells, the distal processes of which unite to form a filament that can be traced into the cavity of the peg.

(ii) *Tuft organs.* These were named and figured by Keilin & Nuttall (1930) but not fully described. In the adult louse there are three tuft organs on the dorso-lateral aspect of the fifth segment and one at the tip of the fourth segment on its outer side. Each consists of a minute cone arising from the floor of a saucer-shaped depression. At the apex of the cone there is a tuft of four tiny delicate hairs which stain weakly with haematoxylin.[1] These hairs appear to arise from a delicate membrane. Below this is a little oval cavity through which runs a deeply staining rod or filament attached at the point where the four hairs unite. A curved tubular thread connects this rod with a group of five or six sense cells (Fig. 28C).

[1] The pits and cones alone are mentioned by Alessandrini (1919).

(iii) *Tactile hairs.* These are of the usual type and consist of a slender bristle arising from a socket below which are trichogen and tormogen cells and a single sense cell with axon fibre (Fig. 28B). They vary somewhat in number, but there are usually 5–7 on segment 1, 8–10 on segment 2, 5–7 on segment 3, 3–4 on segment 4, 3–4 on segment 5.

(iv) *Scolopidial organs.* In segment 2 there are Johnston's organ and some chordotonal organs, which will not be described in detail.

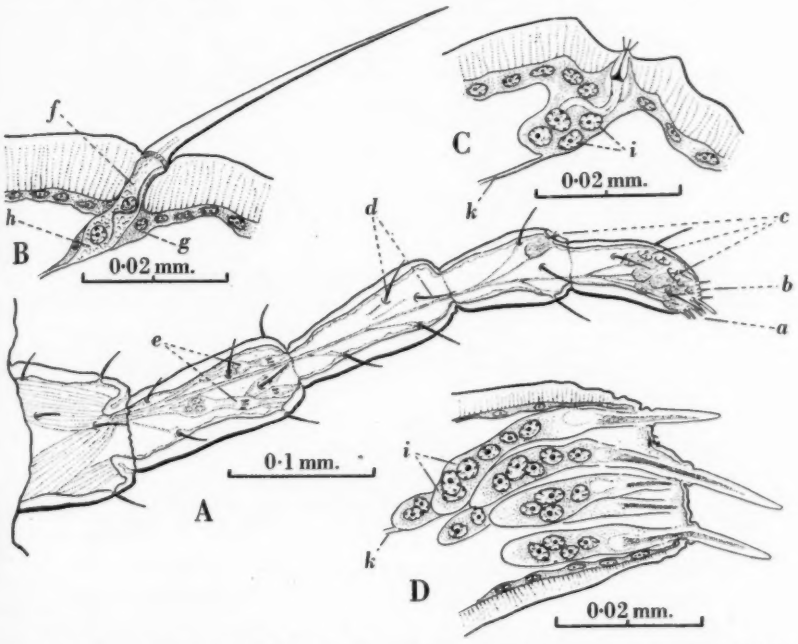

Fig. 28. A, dorsal view of left antenna. *a*, peg organs with rounded tips; *b*, peg organs with sharp tips; *c*, tuft organs; *d*, tactile hairs; *e*, scolopidial organs. B, detail of tactile hair. *f*, trichogen cell; *g*, tormogen cell; *h*, sense cell. C, detail of tuft organ. D, detail of peg organs. *i*, sense cells; *k*, nerve.

Sense organs elsewhere in the body

(i) *Tactile hairs* of the type described are widely scattered over the body. According to Brühl (1871) quoted by Müller (1915) there are about 150 on the whole insect. They are particularly numerous around the mouth parts and on the legs.

(ii) *Campaniform organs.* On the lower surface of each trochanter there are five sense organs, two anterior and three posterior (Fig. 29A) pointed out by Keilin & Nuttall (1930) as a "new type of sensory organ of unknown function". In section these appear to be typical campaniform organs, con-

sisting of a thin dome with a deeply staining rod inserted into it and a sense cell with accessory cells below (Fig. 29B).

(iii) *Chordotonal organs* occur in the femur, tibia and tarsus of each leg. They have been figured by Keilin & Nuttall (1930).

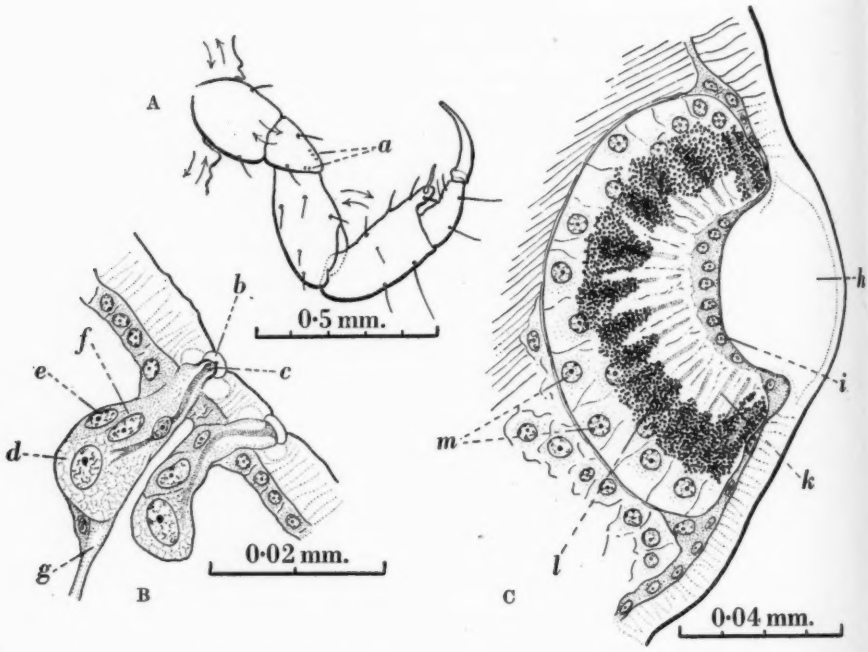

Fig. 29. A, ventral view of leg showing the campaniform organs (*a*) in the trochanter. B, section through these campaniform organs. *b*, dome; *c*, scolopale; *d*, sense cell; *e*, accessory cell; *f*, membrane-forming cell; *g*, nerve. C, horizontal section through eye. *h*, corneal lens; *i*, corneagenous cells; *k*, rhabdoms; *l*, pigment; *m*, nuclei of retinal cells.

Eyes

The eyes lie at the sides of the head and are directed exactly laterally. Fig. 29C shows a horizontal section. The structure, which has been briefly described by Müller (1915), is similar to that of many insect ocelli. Below the biconvex corneal lens is the layer of corneagenous cells, and below these again the cup-shaped retina. This is made up of elongated visual cells with basal nuclei. The rhabdoms occupy the distal two-thirds of these retinal cells; their distal halves are clearly visible in section; but their proximal halves are closely invested by the pigment granules which fill the middle region of the retinal cells.

LOCATION OF THE SENSES

Antennal movements

Lice have been observed under the binocular microscope in a small arena 2 cm. in diameter in which alternative stimuli were offered in the two halves on the same lines as before. As the insect walks the antennae make continuous and more or less synchronous horizontal movements. They are occasionally raised or lowered. Often the head makes slight side to side movements at the same time.

On passing from 30 to 40° C. the antennal vibrations become so rapid that the individual movements cannot be followed. The insect then turns round and retreats.

On coming from 30 to 15° C. the antennal movements become very slow. Often both antennae are extended forwards together. The insect then turns round. It does not usually test the surface by contact with the antennae, though it may occasionally do so.

If the voile floor of the arena rests on the arm, one-half being separated from the skin by metal foil, the antennal movements occur as before. But on the side exposed to the skin the louse occasionally stops and reaches downwards with the antennae. When allowed to walk on the skin it soon stops and probes and sucks blood. During this process the antennae are spread out and in close contact with the skin.

If the floor rests on a warm stage at 30° C. with wet and dry blotting paper below the two halves respectively, the louse often stops dead for a few moments when the antennae *alone* extend into the moist side; it then retreats backwards or turns round.

When one half of the arena is covered with woollen stockinet and half with silk, the same antennal movements take place. As the louse walks it is evident that the antennae and the legs are constantly being stimulated by contact with upstanding fibres in the wool; whereas on the silk, only the tips of the legs are in contact, and the antennae on the rare occasions when they are lowered to the surface.

Senses of smell and humidity

These observations suggest that the senses of smell and humidity are located in the antennae. The antennae were therefore removed from a number of insects proximal to the basal segment, and it was found that reactions to smell and humidity had disappeared. A detailed study of the antennae in relation to these senses was therefore carried out. The humidity sense was tested throughout by offering a choice of 95 % and 76 % R.H. The sense of smell was tested by means of the avoidance of "odourless distillate" (p. 85) to which very few normal insects are indifferent.

In preliminary experiments the fifth antennal segment on both sides was cut through with scissors in a number of lice under ether. In some cases the

segment was only partially removed, but in all the thin-walled sensilla at the tip were eliminated. These insects were tested 18 hr. later. In all of them the reaction to the smell of "odourless distillate" had been eliminated, but in several the humidity reaction still remained. This suggested that the peg organs at the tip are the organs of smell (as Müller (1915) supposed) and that the tuft organs are the organs of humidity. This idea was therefore tested systematically.

The lice were lightly etherized and the sensilla covered with cellulose paint applied by means of the finest entomological pin bent at the point into a minute hook. The insects were in two groups: (i) with the apical peg organs alone covered, (ii) with the dorso-lateral aspect of the fourth and fifth segments covered but the peg organs left exposed. The accuracy of covering was confirmed with the high power of the microscope and those insects discarded in which the paint had not the desired distribution. The insects responded normally 3 or 4 hr. later; the reactions were often confirmed after 24 hr.

Table 1

Treatment	No. of lice	Humidity +	Smell +
Peg organs covered, tuft organs exposed	25	20	1
Tuft organs covered, peg organs exposed	20	0	19

Table 1 summarizes the results obtained and Fig. 30 gives two typical examples. Five out of twenty-five insects were indifferent to humidity although the tuft organs were exposed, and one out of twenty was indifferent to the smell of "odourless distillate" when the peg organs were exposed; but the results strongly support the conclusion that the tuft organs are sensitive to humidity and the peg organs to smell. There was one exception in which the peg organs were covered and the insect still reacted quite definitely to odourless distillate. The covering in this case was certainly complete, the result is therefore unexplained; but the covering was exceedingly thin at some points; possibly the odour penetrated through this thin layer to the sense organs.

An attempt was made to see if the tuft organs show any visible change when exposed to alterations in humidity. Lice killed with chloroform were placed in the small gas chamber previously described (Wigglesworth, 1930, 1935) and air first at 0 % R.H. and then at 95 % R.H. passed over them while the tuft organs were watched under the 4 or 2 mm. objective of the microscope. No movements in the fine hairs of the tuft could be detected with certainty.

In few insects has the humidity sense been located. Pielou (1940) has shown that in the adult mealworm beetle *Tenebrio* it is strictly confined to the antennae, and by amputation of the antennae at different levels has concluded that the pit peg organs and peg organs are the sensilla responsible. These are also regarded as the true olfactory organs. The results therefore suggest that the apparent response to humidity in this insect may perhaps be a response

to the altered perceptions of the olfactory organs in the drier or moister air; or alternatively, that changes in the humidity of the air may so affect the thin-walled olfactory sensilla as to serve as stimuli to them. In spiders, also, Blumenthal (1935) concludes that the "tarsal organ", a deep pit with one or several peg organs arising from the floor, responds to both humidity and smell.

In *Pediculus*, however, the two senses seem quite distinct. The "tuft organs" are an entirely new type of humidity receptor. The rounded "peg organs" on the other hand, are the commonest type of olfactory organ. Whether the sharply pointed organs have a different function has not been determined (cf. p. 98).

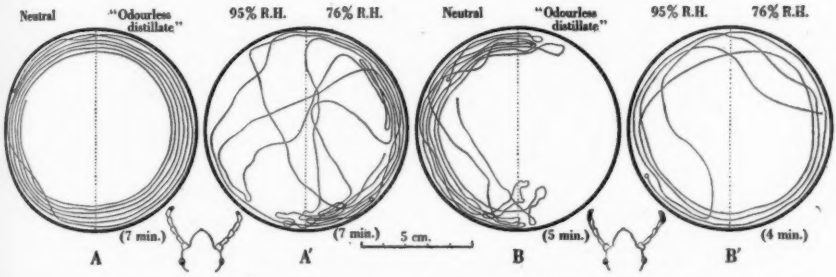

Fig. 30. A, A′, reactions of louse, with peg organs alone covered, to smell and humidity. B, B′, reactions of louse, with the tuft organs alone covered, to smell and humidity.

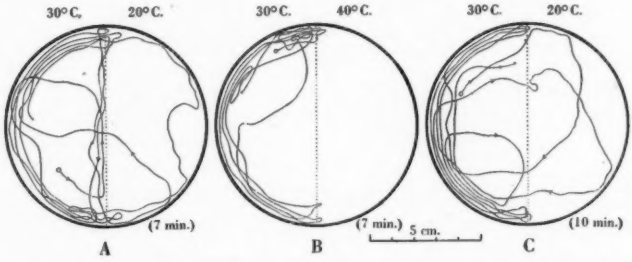

Fig. 31. A, B, reactions to temperature in lice deprived of both antennae. C, reaction to temperature in louse deprived of both antennae and anterior part of head.

Sense of temperature

The temperature sense is not confined to the antennae. Fig. 31A shows that in a louse in which the antennae had been removed 24 hr. previously, exposed to 30° C./20° C., the avoidance of 20° C. may be as efficient as in the normal insect. And Fig. 31B shows efficient avoidance of 40° C. in a louse similarly treated. Even when the antennae are removed and the head cut through with scissors in front of the antennal sockets (the wound being sealed with paraffin) there may be complete avoidance of 20° C. (Fig. 31C).

Thus the sense organs responding to temperatures above and below the optimum certainly occur elsewhere besides the antennae. The effect of removing the antennae on the delicacy of the responses has not been systematically tested. But the sensitivity to high temperatures at least is certainly reduced. Thus in the normal louse avoidance of 40° C. is almost complete; but in lice without the antennae it is absent more often than not. Homp (1938) likewise observed that lice deprived of their antennae come closer to a very hot object; and Weber (1929) noted that *Haematopinus* without the antennae wander more widely in a temperature gradient, particularly at the hot end.

The temperature sense therefore resides probably in the antennae as well as elsewhere; but the sense cannot be ascribed to any particular sensilla. In

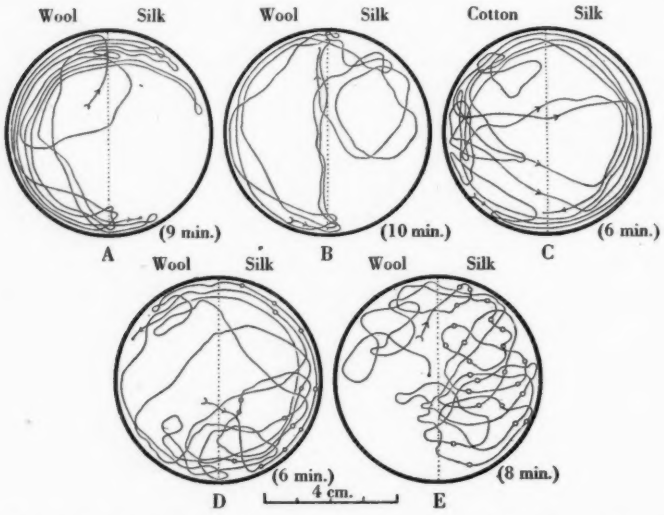

Fig. 32. Reactions to contact in lice deprived of both antennae. In B the anterior part of head also removed. The circles in D and E indicate the spots where the louse stopped and probed.

the bug *Rhodnius*, in which there is a very delicate temperature sense confined to the tips of the antennae, there is some evidence that the sense organs concerned are the innumerable finely pointed, relatively thick-walled hair sensilla (Wigglesworth & Gillett, 1934*a*). It is just possible that the pointed members of the group of peg organs on the fifth antennal segment have this function in *Pediculus*.

Sense of contact

We have seen that when the louse moves on rough material the antennal hairs are constantly being stimulated. But after removal of the antennae (Fig. 32A) or of the antennae plus the anterior part of the head (Fig. 32B) the preference for woollen stockinet as compared with silk still persists; although,

as in the normal louse, this response may disappear in the course of an experiment.

A response seen several times in the louse without antennae, though never in the normal insect, was a preference for the smoother material (Fig. 32C). This is probably associated with the feeding reaction. Homp (1938) observed and I have confirmed that lice without antennae will repeatedly probe a warm smooth surface, whereas intact lice nearly always need the additional stimulus of smell.[1] Thus Figs. 32D, E, show the tracks of lice without antennae, which had an obvious preference for the silk. The points where they stopped and probed the surface are indicated by circles. Later these insects became indifferent and ceased probing.

In these reactions to contact, therefore, organs all over the body are concerned, and orientation by contact stimuli is possible without the antennae.

Function of chordotonal and campaniform organs

These are generally regarded at the present time as proprioceptive organs recording the position of the appendages or the strains set up in them by their own movements (Wigglesworth, 1939).

We shall see that the appreciation by the insect of its own antennal movements is probably an important factor in the orientation of the louse at the boundary of a steep gradient of humidity or smell. The Johnston's organ and chordotonal organs in the second antennal segment probably serve this function.

In the movements of the legs, abduction and adduction occurs proximal to the coxa; flexion occurs chiefly between coxa and trochanter and between femur and tibia (Fig. 29A). The campaniform organs, lying ventrally and laterally at the distal end of the relatively fixed trochanter, are well placed to detect strains in the limb caused by any resistance to these movements (cf. Pringle, 1938).

MECHANISMS OF ORIENTATION

Introduction

We must now attempt to define the mechanisms by means of which the louse orientates itself in relation to the various stimuli we have been considering.[2]

Simple mechanisms of orientation are classified as: (i) *kineses*—effects exerted by stimuli on the *rate* of random movements of the animal, and (ii) *taxes*—movements which result from a discrimination of the *direction* of stimulation.

[1] Homp calls attention to the analogous behaviour of ticks which will feed on any host if their Haller's organ is removed (Hindle & Merriman, 1913). A somewhat similar response is shown by *Rhodnius*, which will probe moving objects of all kinds when deprived of the senses of temperature and smell by removal of the antennae (Wigglesworth & Gillett, 1934*a*).

[2] In this section I have made extensive use of the modified classification of mechanisms of orientation drawn up by Fraenkel & Gunn (1940). I am greatly indebted to Dr D. L. Gunn for allowing me to use the proofs of this work in advance of publication.

Kinesis may be subdivided into (*a*) simple effects of the intensity of stimulation on the rate of movement (*orthokinesis*), and (*b*) effects on the frequency of turning, that is, on the rate of change of direction of movement (*klinokinesis*).

Taxes or directed movements may be subdivided into (*a*) movements whose direction is dependent on the comparative intensities of stimulation acting simultaneously on bilateral sense organs (*tropotaxis*), and (*b*) movements whose direction is dependent on the comparison of intensities of stimulation on each side by regular deviations of the body or the antennae; that is, by the comparison of intensities which are successive in time (*klinotaxis*).

In the past klinokinesis and klinotaxis have been included together under the terms "avoiding reaction", "trial and error" or "phobotaxis" (Kühn, 1919; Fraenkel, 1931); but the fact that in the former the movements are random, while in the latter the movements are directed, is considered by Fraenkel & Gunn (1940) a sufficient reason for their separation.

In the movements of an animal the mechanism of orientation may of course change from moment to moment; and even at the same instant more than one mechanism may be operating.

Orientation to light

Sudden exposure to bright light causes an arrest or reduction in the movements of the louse (p. 89). This is an example of negative "orthokinesis". Acting alone it would tend to keep the louse exposed to the light; but since this insect remains chiefly in darkness, it cannot be an important element in the light response.

Some individuals exposed to a bright light begin to change direction frequently and follow a convoluted course (Fig. 25). This is an example of "klinokinesis". As will be seen below it will tend to restore the insect to the dark.

It is obvious, however, that orientation to light is primarily a directed response determined by the comparison of the light coming from different directions. If one eye is covered with black cellulose paint and the louse is then exposed in an arena uniformly lit from above so as to give a surface illumination of 500 metre candles, it circles continuously towards the covered side (Fig. 33). It is clearly the simultaneous comparison of stimulation in the two eyes which is important in determining the direction of movement—an example of "tropotaxis". As in other insects, the circus movements become progressively weaker as the exposed eye becomes adapted to the light; they have generally ceased in half an hour or so. The circus movement is much more pronounced in an arena with white walls (Fig. 33A); in an arena with black walls the louse tends to cling to the dark surface (Fig. 33A′); indeed the dark wall may eliminate the circus movement altogether (Figs. 33BB′).

These results emphasize once more the importance of horizontal light in

the orientation of the louse. This is doubtless correlated with the position of the eyes, which are exactly lateral.

The orientation of an insect to a relatively small dark object (Figs. 27C, D) is sometimes termed "skototaxis". Probably, however, in the louse at least, it is merely another manifestation of the tropotactic response. The formation of images by the eyes must be almost non-existent; but the dark spot in the horizontal plane will reduce the total light stimulation in one visual field so that the insect is deviated towards it. The effect naturally increases as the object comes nearer (cf. Fig. 27D).

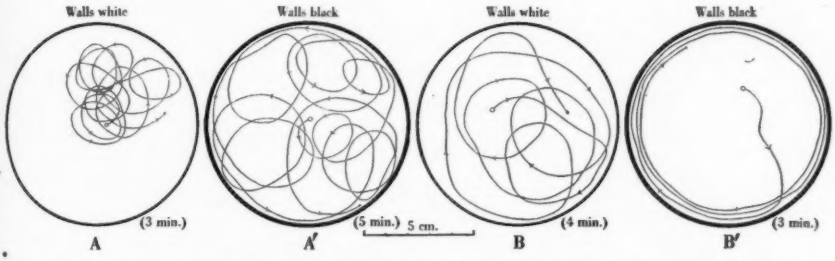

Fig. 33. Circus movements in lice with one eye covered. A, left eye covered, walls of arena white. A′, the same, walls of arena black. B, right eye covered, walls of arena white. B′, the same, walls black.

Orientation to smell and humidity

The methods of orientation to these two stimuli agree in almost every respect; they may therefore be considered together. On entering a mildly unfavourable region (Figs. 16B, C; 20B, C) the louse no longer walks straight round the periphery of the arena but follows a convoluted course, changing its direction repeatedly. This is an example of "klinokinesis". Under the conditions of experiment here used it may cause the insect to remain much longer than it otherwise would on the unfavourable side. But after a time it becomes adapted to the weak adverse stimulus; it then goes straight once more and so regains the favourable side.

If the louse is more reactive or the adverse side of the arena more repellent, the insect may turn round instantly on reaching the boundary or may walk backwards (Fig. 10B; 17B; 20A). This is the limiting case of "klinokinesis"; it is often referred to as the "shock reaction" or "avoiding reaction". But as Ullyott (1936) points out in the case of the planarian *Dendrocoelum*, and as is obvious from an inspection of the tracks of lice given here (e.g. Fig. 10C; 16C), there are all intermediate stages between the two reactions—if the insect turns soon enough and sharply enough it will regain the favourable side; if the turn is delayed or insufficient it may remain in the adverse half of the arena.

It is obvious in some experiments, however, that the response at the

boundary, where there is a steep gradient in the intensity of stimulation, is a *directed response*. This is most evident when the insect approaches the boundary obliquely. It is true that it may occasionally turn into the adverse zone—as would be expected if the direction of turning were entirely random as in "klinokinesis"—but in the vast majority of instances the louse inclines away from the boundary and back into the favourable side. Fig. 10B is a typical example.[1]

What is the nature of this directed response? If it is by tropotaxis, the elimination of the sense organs on one side ought to cause the insect to turn always towards this side on reaching the boundary or to circle always to this side in the adverse half of the arena.

Eight lice were lightly etherized and the fifth antennal segment and the terminal half of the fourth segment covered with cellulose paint, on the right side in four insects, on the left side in four more. They were then exposed in an arena with "odourless distillate" below one half; their tracks were copied

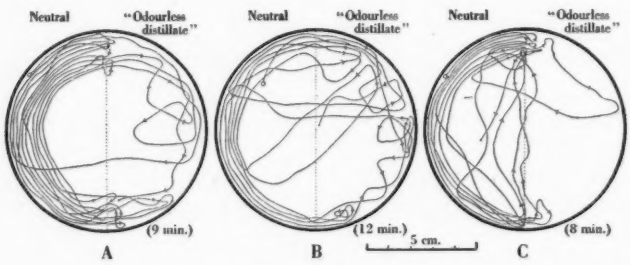

Fig. 34. Tracks followed by lice, with the organs of smell on one side covered, in the presence of a repellent odour. A, right antenna covered; B, C, left antenna covered.

and the number of turns made in each direction observed. As can be seen in Fig. 34 they turn indifferently towards the exposed or the covered antenna. This is so during the abrupt turns at the boundary (Fig. 34A) and during the sinuous movements after entering the adverse side (Fig. 34B). Out of a total of 116 turns[2] made in the course of these records sixty were towards the covered side, fifty-six towards the uncovered side. In tests with 95 %/76 % R.H., out of forty-three turns in the adverse half, 23 were towards the covered antenna, twenty towards the exposed.

In these experiments, with the sense organs on one side completely out of action, the difference in stimulation on the two sides must be far greater than anything encountered by the intact insect. Yet this has no significant effect on the direction in which the louse turns on encountering an adverse stimulus.

[1] Hase (1915) has described the pursuit of the finger by the louse "like a hound on the trail"; here also the orientation appears to be directed.

[2] Each deviation from the normal straight course has been counted as a turn—whether this causes the insect to go through 180° and reverse its direction or whether it merely causes it to incline 90° from its previous course.

Further, it is evident, from Fig. 34C for example, that the louse with a single antenna still retains the faculty for directed orientation at the boundary.

One must therefore conclude that this orientation is effected not by the comparison of simultaneous stimuli in the bilateral sense organs (tropotaxis), but by comparison of stimuli at successive moments (klinotaxis). Gunn & Pielou (1940) obtained similar results in the orientation of *Tenebrio* to humidity. In this reaction the swinging of the antennae and head from side to side (p. 95) is doubtless important. The scolopidial organs in the second antennal segment probably serve as proprioceptors to indicate the position of the antennae at the moment of greatest stimulation (cf. Sioli (1937) on *Cimex*). A similar method of orientation was described in *Rhodnius* (Wigglesworth & Gillett, 1934*a*); but here the long antennae are moved independently, so that the orientation can be effected by the insect at rest.

An important factor in the behaviour of the louse is the adaptation shown in a constant field of stimulation. As Ullyott (1936) has pointed out, if the animal merely went straight in a favourable zone and followed a sharply convoluted course on entering an adverse zone, this would result in its being trapped in the unfavourable region. In fact it gradually becomes adapted, makes increasingly long excursions before turning (unless it should chance to enter a still more unfavourable region) and finally goes straight. On reaching a favourable zone it continues to go straight.

This adaptation to an adverse field of stimulation is well seen in Fig. 10C. In the case of the high humidity of 95 %, the "adaptation" may be so complete after prolonged exposure that the response is reversed and turning may occur on entering the 76 % R.H. side. Indeed, what constitutes an "adverse" region depends to a large extent upon the experiences in the immediate past; that is, on the state of adaptation. Blotting paper or smooth silk cause increased turning only if the louse has been on rough material (p. 87); clean voile constitutes a favourable zone if the other half of the arena contains "odourless distillate"; it forms an adverse zone and induces active turning if the other half lies above man-scented cloth (pp. 83, 85). The response may be regarded as an elementary form of memory.

Orientation to temperature and contact

Orientation to temperature is clearly of the same kind as to humidity and smell; that is, "klinokinesis" (Figs. 3, 4). Homp (1938) likewise concluded that this orientation was chiefly "phobotactic". But she suspected that there was a directed element in the response when close to the source of stimulus; and on the assumption that the antennae are the chief site of the temperature receptors she removed the antennae from one side in twelve lice. When these were exposed to a uniform temperature of 29° C., seven of them turned more frequently towards the intact side, five towards the operated side. She interprets these results as indicating "tropotaxis", but the figures are quite unconvincing.

I have repeated the experiments and obtained the same results. Removal of one antenna is very liable to induce a bias and cause the insect to circle in some cases towards and in some away from the injured side. But, allowing for these insects, no evidence could be obtained that the louse turned more frequently away from the intact antenna upon entering an adverse temperature of 20 or 40° C., or towards the intact antenna in a favourable temperature of 30° C.

The directed orientation that occurs along the boundary between the temperatures is therefore again probably "klinotactic". As we have seen (Fig. 31A) it may be shown by lice deprived of both antennae. Tropotaxis would be more likely to occur in response to radiant heat. But as we have seen (p. 76) within the range of temperatures employed the louse does not react to radiant heat.

The mechanism of *orientation to contact* appears to be the same as that to the other stimuli. It consists of an increased frequency of turning upon a smooth surface (klinokinesis), provided that the insect has recently experienced a rough surface. Combined with this is an orthokinetic effect—the louse moves more actively and is less disposed to settle down and come to rest on a smooth surface.

Sensory responses and the normal environment

The warmth, the smell and perhaps the smooth surface of the skin are important factors in inducing the feeding response in the louse. But the reactions dealt with in this paper are those shown by fully gorged lice; they are the responses concerned with keeping the louse within its normal environment.

These responses have an obvious bearing on many familiar facts about the habits of the human louse. The optimal zone of temperature, 28–31° C., is that which exists between the skin and the clothing (Martini, 1918). The humidity below the clothing in men at rest is rather low, 23°–70 % R.H. (Mellanby, 1932; Marsh & Buxton, 1937); it will increase in the outer layers of the clothing as the air is cooled, and will increase during sweating. It has been observed by Dr John MacLeod and Dr H. J. Craufurd-Benson (personal communication) that the number of lice on the inner garments of infested labourers decreases markedly after work entailing profuse sweating; and Nuttall (1917) has attributed the reduction in the number of lice in summer, at least in part, to the increased moisture beneath the clothing. Lice tend to leave a patient with fever (Lloyd, 1919); here the high temperature and increased humidity may both operate. The preference of *Pediculus* for the smell of man (p. 83) is in accordance with the rarity with which this louse is found on other hosts. The attraction to rough materials, and to the smell of other lice and of their excreta explains the aggregation of lice in the seams of clothing. And the orientating effect of light reaching the eyes horizontally

explains the comparative rarity with which the louse strays onto the exposed parts of the body.

But beyond these obvious uses of the reactions studied the experiments throw some light on the methods by which the louse reaches environments favourable to it. Fig. 35 is the hypothetical track of a louse approaching a centre from which emanates some favourable diffuse stimulus of temperature, smell or humidity. The louse has become adapted to the unfavourable environment and therefore goes approximately straight. On entering the zone which provides the favourable stimulus it shows *no* response. But once having experienced this stimulus it makes turning movements as it leaves the zone again. Thus the insect, turning when the intensity of stimulus falls, keeping

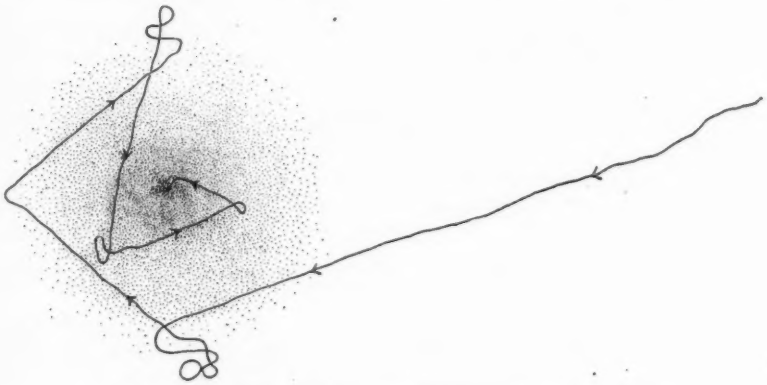

Fig. 35. Hypothetical track of louse approaching the centre of some favourable diffuse stimulus.

straight while the stimulus remains constant or increases, is led to the centre of the favourable zone. When the gradient is steep, directed responses effected by the comparison of stimulation in each direction in turn are also added (p. 103).

In this way the louse will be led by successive stages into those sites where the stimuli of temperature, smell, humidity and contact are optimal. In respect to light, the same mechanism operates to some degree; but here the directed element in the stimulus is far more important; it is particularly effective when the louse is already in the dark (p. 90).

Finally, it may be emphasized once more that, except perhaps in a very steep gradient, the louse shows no sign of being *attracted* by a favourable stimulus. It simply makes turning or avoiding movements when such a stimulus ceases to operate; just as it makes avoiding movements on encountering a stimulus that is repellent.

Summary

(i) *Sensory responses*

The reactions of the body louse to temperature, humidity, smell, contact and light have been tested in an arena divided into two halves.

Temperature. A temperature of 29–30° C. is preferred before 32° C. or 27° C. As the alternative temperature rises above 32° C. or falls below 27° C. the avoidance becomes increasingly strong. Different individuals vary in sensitivity.

These results are in accordance with those observed in a linear gradient of temperature, in which the lice collect chiefly in the region from 28 to 31° C.

The response is always to air temperature; there is no response to radiant heat from objects at 20–45° C.

Humidity. The louse is generally indifferent to humidity over the range from 10 to 60 or 75 % R.H. Higher humidities are avoided. But when offered two humidities the choice is greatly influenced by the conditions experienced by the louse in the immediate past; it avoids any change; hence different individuals may show quite different responses. Moreover, when offered the choice of very moist air (95 % R.H. or over) and very dry (47 % R.H. or under) the louse becomes more readily adapted to the moist air and begins to avoid the dry.

Smell. The louse prefers cloth that has been in contact with human skin to clean cloth or cloth smelling of dog or rabbit. The smell of other lice and of their excreta is also attractive. Many substances serve as repellents; a refined petroleum with a very faint odour has been chiefly used for the experiments.

Contact. When offered smooth and rough materials the louse chooses the latter. It moves more rapidly on smooth materials and does not come to rest so readily. It shows little response to air currents unless very strong, when they are avoided.

Light. The movements of the louse are arrested or retarded by sudden exposure to a bright light, and sometimes it may show avoiding movements. But the movement of the louse towards dark places is mainly a response to directed light received by the horizontally placed eyes. Slight differences in the light received from different directions exert a much greater effect if the louse is exposed to a low level of general light intensity.

The movement of the louse towards relatively small dark objects is probably a manifestation of the same response.

(ii) *Sense organs*

The antenna bears three types of sensillum. (i) *Tactile hairs* on all segments. (ii) *Peg organs* at the tip of the fifth segment; these are shown to be olfactory receptors. (iii) *Tuft organs* on the fourth and fifth segments; these are shown

to be humidity receptors. There are also a Johnston's organ and chordotonal organs in the second antennal segment.

Tactile hairs occur around the mouth parts, and on the legs, etc.; *chordotonal organs* in the femur, tibia and tarsus of each leg; and there is a group of five *campaniform organs* on the lower surface of each trochanter.

The *eyes* are described.

The temperature sense is widely distributed over the body; orientation to high or low temperatures still occurs after removal of the antenna and the anterior half of the head, although the sensitivity is reduced.

(iii) *Mechanisms of orientation*

The mechanism of orientation to the diffuse stimuli of temperature, humidity, smell and contact is the same. It consists in an increase in random turning movements upon entering a zone of adverse stimulation (phobotaxis or klinokinesis). This may result in an immediate return to the favourable zone if the response is strong and immediate, or in a long convoluted course in the unfavourable zone if the response is weak or delayed.

Sensory adaptation is very important in this response. For the increased rate of turning disappears after prolonged exposure to the unfavourable stimulus and only appears again after a favourable stimulus has been experienced.

There is no evidence that the louse is "attracted" by a favourable stimulus. It shows only an avoidance of zones where a "repellent" is present or where a favourable stimulus (recently experienced) is absent.

Where there is a steep gradient between the adverse and favourable zones the louse may show a directed orientation. This appears to be brought about by a comparison of successive stimulation to right and left by swinging the body and antennae from side to side (klinotaxis). There is no evidence that the comparison of simultaneous stimulation in the antennae (tropotaxis) plays any part.

In orientation towards darkness, increased turning in a bright light (klinokinesis) plays a small part. The comparison of stimulation by horizontal light received in the two eyes (tropotaxis) is far more important. If one eye is covered the louse makes circus movements towards this side.

The relation of these responses to the biology of the louse is discussed.

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SOME NOTES ON THE RELATIONSHIP OF PLANT VIRUSES WITH VECTOR AND NON-VECTOR INSECTS

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(With Plates III and IV)

The survival of both plant and animal viruses in the bodies of non-vector insects has been recorded on several occasions. Bennett & Wallace (1938) have shown that the virus of sugar-beet curly-top can be recovered from the aphis *Myzus persicae* up to 14 days after ingestion and up to 21 days from the leaf-hopper *Aceratagallia californica*. Again, the mosquitoes, *Aëdes* spp., *Mansonia* spp. and *Haemagogus* spp., have been shown (Whitman & Antunes, 1937) to retain the virus of yellow fever in their bodies for different periods. Yet none of these insects can act as vectors of the viruses in question.

Inhibition of infection. With one exception (McClintock & Smith, 1918) there is no record of a sap-transmissible virus being isolated from the body of an infective insect vector by mechanical means though the attempt has been made several times (Storey, 1926; Smith, 1929; Fukushi, 1934). The reason for this failure has now been shown to be due to the presence of an inhibitor in the body of the insect (Black, 1939; Smith, 1939) which prevents infection of the plant by the virus. This effect may be analogous to that of trypsin as described by Stanley (1934). Black, working with the virus of tobacco mosaic and such insects as aphides and leaf-hoppers, has shown that the virus can be separated from the inhibitor by means of filtration or ultracentrifugation. Since Black has dealt with this question at length in his paper it is not proposed to do more than mention a few additional points.

In these experiments the virus used was that of tobacco necrosis (Smith & Bald, 1935), and the insects were the large tobacco "hornworm" (*Protoparce sexta*) and other caterpillars. The test plant for the virus was the bean (*Phaseolus vulgaris*). Extracts of these caterpillars when mixed with fairly concentrated virus samples completely inhibited infection of the test plants. Similar inhibition of infection was obtained by mixing the virus with extracts of a variety of miscellaneous insects, with the blood of earthworms and with the blood and tissues of fish, and it is probable that any animal protein would have this effect. Some inhibition of infection was also obtained by mixing with the virus plant proteins obtained from beans. No protection was apparent, however, when the leaves were first rubbed with the plant extract before inoculation with the virus.

In experimenting with the insect juices, it was found that it made no difference whether virus and inhibitor were mixed together before inoculation to the test plant or whether the leaf was first rubbed with the inhibitor and then

inoculated with the virus. In both cases the protection was complete. Moreover, it appeared that the inhibitor still had the property of reducing infection even if rubbed over a leaf immediately *after* the leaf had been inoculated with a concentrated virus suspension. In such a case the number of lesions was reduced by about 80%.

Black has stated that the inhibitor from his leaf-hoppers remained in the supernatant after spinning in the ultracentrifuge. This observation has been confirmed with the inhibitor from earthworms and caterpillars. Pl. III shows the complete inhibition obtained with the supernatant fluid from extracts of earthworm after spinning for 2½ hr. at 30,000 r.p.m.

Boiling the inhibitor reduced its protective action to a very large extent but did not completely eliminate it (Pl. IV). Attempts to separate inhibitor from the tobacco necrosis virus by means of ultrafiltration were not very successful. The experiments with the ultracentrifuge suggest that the inhibitor is smaller than the virus since it did not sediment after 2½ hr. spinning. Nevertheless, the inhibitor is not easily filterable and barely passes a membrane of an average pore diameter large enough to let the tobacco necrosis virus through. This may perhaps be explained by assuming adsorption of the inhibitor on the membrane.

Recovery of virus after ingestion by a non-vector insect. In these studies on the survival of plant viruses in non-vector insects extensive use was made of the same large sphinx moth caterpillar previously mentioned. These caterpillars feed readily upon the tobacco plant which is suceptible to the different viruses used in the experiments and ingest enormous quantities of virus.

Two aspects of the relationship between the virus and the non-vector insect have been studied, first as to whether the insect actually destroys the virus by its digestive processes and secondly whether the virus enters the blood and survives there for a period of time.

Five plant viruses were used in the experiments, four are sap-inoculable and the fifth cannot easily be transmitted in this way. They were *Nicotiana virus* 1 (tobacco mosaic virus); *Nicotiana virus* 11 (tobacco necrosis virus); *Nicotiana virus* 12 (tobacco ringspot virus); *Solanum virus* 1 (potato virus *X*); and *Beta virus* 1 (sugar-beet curly-top virus).

To ascertain whether the virus was digested or otherwise inactivated within the insect, the caterpillars were fed on Turkish tobacco plants infected with different viruses, and the faeces were then collected and tested for virus by inoculation to various suitable plants. Preliminary tests showed that unlike extracts of the caterpillar itself there was no inactivation or inhibition of the virus produced by the faeces themselves. In order to test for the presence of the curly-top virus in the faeces it was necessary to feed the leaf-hoppers, *Eutettix tenellus*, on a sweetened suspension of the faeces by means of the artificial feeding technique described by Bennett (1935).

It was found that three of the viruses, *Solanum virus* 1, *Nicotiana virus* 12, and *Beta virus* 1, could not be recovered and so were presumably inactivated. *Nicotiana virus* 11 was recovered in very small amounts on two occasions while

Nicotiana virus 1 was present each time in the faeces but in greatly reduced concentration. It seems as if the virus must be highly concentrated to avoid complete inactivation within the body of the caterpillar. It was thus possible to separate *Nicotiana virus* 1 from a complex of viruses by passing them through the caterpillar.

The second point to be investigated was the question whether the virus entered the blood of the insect after the latter had fed on a virus-infected plant. All attempts to demonstrate the presence of *Nicotiana virus* 1 and the other sap-inoculable viruses in the blood were unsuccessful, and this is not surprising since the presence of the inhibitor would be sufficient to prevent infection. Recourse was had, therefore, to the curly-top virus, and it is paradoxical enough to hope to get this information by the study of a non-sap-transmissible virus and the complications of technique that this involves. By so doing, however, the action of the inhibitor is avoided, since this does not come into play with insect-transmitted viruses. In other words by feeding extracts of the blood of the experimental insects to the specific insect vector of the virus the necessity for the ultracentrifuge or ultrafiltration is cut out since the vector itself does the necessary separation of virus and inhibitor.

In these experiments twelve caterpillars were fed on Turkish tobacco plants infected with the curly-top virus. After a week or ten days' feeding the caterpillars were bled and the blood was fed to the leaf-hoppers. This experiment was completely negative and a second similar experiment gave the same result.

One explanation of the apparent failure of the viruses to pass from the alimentary canal of the caterpillar into the blood may be that the peritrophic membrane which lines the interior of the gut presents an impermeable barrier to the movement of the virus.

Injection of virus into a non-vector insect. The next step was to investigate the fate of the virus in the caterpillar when artificially introduced. Because of the action of the inhibitor it was necessary to use a virus with a specific insect vector which would automatically separate the virus from the inhibitor. The curly-top virus was used and it was prepared as follows: An alcoholic suspension was made of viruliferous leaf-hoppers and the precipitate centrifuged off and suspended in 0·5% saline. In these experiments caterpillars about two-thirds grown were employed and the virus was injected into the blood just below the skin, care being taken to avoid puncturing the alimentary canal. A fine hypodermic needle was used and four injections were made into each caterpillar using 0·5 c.c. of virus on each occasion. If the injections were made carefully the caterpillars as a rule suffered no ill effects though a small proportion died of septicaemia. Twenty-four hours later the injected caterpillars were bled and the blood treated in two ways. To obtain the blood the caterpillars were anaesthetized and a prick made in the skin with a fine needle; the caterpillar was then suspended over a dish and the blood collected. In one set of experiments the blood was precipitated with alcohol before being fed to the leaf-hoppers and in

the other it was used unprecipitated. The precipitate was dried and resuspended in 0·1% sodium citrate and also in sugar water while the unprecipitated blood plus a little sugar water was fed direct to the leaf-hoppers. In the first experiment two plants out of ten were infected with the curly-top virus by leaf-hoppers which had fed on the precipitated blood and one out of ten by leaf-hoppers which had fed on unprecipitated blood. In the second experiment two plants became infected from the unprecipitated blood and one from the precipitated; in the third experiment one plant only from the unprecipitated blood became infected.

The results of these experiments indicate that the curly-top virus can persist in the blood of certain caterpillars when introduced artificially.

Since, as shown already, *Beta virus* 1 is apparently inactivated within the body of the caterpillar it was not possible to ascertain if this virus entered the alimentary canal when injected into the blood. It was therefore necessary to use *Nicotiana virus* 1 which is not entirely inactivated by the digestive processes of the caterpillar. Half-grown larvae were injected with 2 c.c. of a concentrated suspension of *Nicotiana virus* 1, and these were then tested for virus in three ways. First, inoculations were made to *Nicotiana glutinosa* with the virus-injected blood of the caterpillars. As was to be expected this experiment was completely negative, since the presence of the inhibitor in the blood was in any case enough to prevent infection. Secondly, the faeces of injected caterpillars were collected and inoculated to appropriate test plants; no infections were obtained with any of the inoculations. Finally, the caterpillars with their blood presumably loaded with virus were fed upon healthy plants of Turkish tobacco, again without infection resulting.

Recovery of Beta virus 1 *after ingestion by flea-beetles.* A number of flea-beetles, exact species undetermined, were allowed to feed for 12 days on sugar-beet plants affected with curly-top. After this period the beetles were crushed and extracted with water, the extract being precipitated with alcohol and resuspended in sugar water for feeding to the leaf-hoppers. In two such experiments five sugar-beet plants, out of a total of thirty colonized with the leaf-hoppers, developed curly-top. A further test, using the faeces of these beetles and also of the cucumber beetle *Diabrotica* 12-*punctata*, gave negative results.

Demonstration of virus in the salivary fluids of viruliferous insects. Since the early work of Carter (1928) no very conclusive evidence has been presented that virus is actually in the saliva of viruliferous insects, although it has always been assumed that the saliva is the vehicle of transfer. The following experiments were therefore carried out to test this point. Using the artificial feeding technique described by Bennett (1935), a number of leaf-hoppers (*Eutettix tenellus*) known to be infected with the curly-top virus were fed on drops of sugar solution. After the insects had been allowed to feed for 3 hr., the drops were transferred to a fresh membrane and fed to a series of known non-infective leaf-hoppers. After an appropriate period these leaf-hoppers were

tested on healthy beet seedlings, one insect to each seedling. In this experiment six out of nineteen seedling beets developed the curly-top disease.

Two additional tests were then made; in these the sugar solution on which the viruliferous leaf-hoppers had fed was evaporated to about half its original volume. Twenty non-viruliferous leaf-hoppers were allowed to feed on each lot and then caged singly on seedling beets. Of the twenty plants inoculated in each test, sixteen plants were infected in one test and thirteen in the other. The apparent concentration of virus in this material is rather surprising in view of the evidence that leaf-hoppers are able to retain virus for months, and it would be of interest to know how they manage to give off so much virus and yet retain it for long periods.

These experiments seem to prove that the saliva of the insect is the medium in which the virus is transmitted.

Discussion

Owing to the complication of the virus inhibitor present in insect tissues, the study of the relationship between plant virus and non-vector insect is a difficult one. The experiments described in this paper suggest that the majority of plant viruses are rapidly inactivated, probably by digestion, within the body of the caterpillar. On the other hand, evidence is offered that some of the more resistant viruses can persist for some time in the blood of the same caterpillar.

It is not to be expected that a caterpillar would be able to infect a healthy plant with curly-top even though the virus was present in the blood, because it is necessary for the virus causing this disease to be injected directly into the phloem for infection to take place. This is not possible with a leaf-eating insect such as a caterpillar. On the other hand, it is curious that such an infectious sap-transmissible virus as that of tobacco mosaic should not be transmitted by the caterpillar with its blood heavily loaded with a concentrated virus suspension. It is known from the work of Storey (1932) and others that it is necessary for virus, ingested by an insect, to pass through the wall of the alimentary canal into the blood before that insect can transmit the virus. But is that the only obstacle to virus transmission? There seem to be cases where an insect still cannot transmit even with virus in its blood. The caterpillar injected with tobacco mosaic virus is an example of this. Furthermore, the work of Bennett & Wallace (1938) has shown that two types of sucking insect, a leaf-hopper and an aphis, both retain the virus of curly-top for as long as 14–21 days but are still unable to transmit it, and in one at least of these two cases the virus seems to be in the blood.

There is therefore another barrier to successful virus transmission by non-vector insects even after the virus has reached the blood of the insect. Does this barrier lie in the salivary glands? Bennett (*in litt.*) has made the interesting suggestion that certain viruses which are not insect-transmitted, such

as those of tobacco mosaic and tobacco necrosis, may be inhibited by lower concentrations of animal protein than viruses which are insect-borne. In this case the salivary deposits introduced into the cells during insect feeding may be a determining factor in insect transmission. In other words such a virus as that of sugar-beet mosaic which is aphis-borne may be less sensitive to the inhibitory action of the accompanying saliva of the transmitting insect than viruses which are not insect-borne. Bennett offers some evidence in support of this thesis from experiments on the effect of insect protein in inhibiting the mechanical transmission of insect-transmitted viruses. Thus he finds that quantities of 50 mg. of *Aphis rumicis* in 1 c.c. of mixture in which the remaining part was juice from mosaic beets produced no noticeable reduction in infection. No infection was produced when 100 mg. per c.c. were used, but with this concentration the inoculated leaves were badly burned. With *Eutettix tenellus*, 100 mg. of leaf-hopper material in 1 c.c. of mixture did not reduce infection.

On the other hand, Black (1939) reports inhibition of infectivity by insect juices of the viruses of potato yellow dwarf and turnip mosaic, both of which are insect borne.

SUMMARY

Extracts of caterpillars and other insects are shown to inhibit the infective power of tobacco mosaic and tobacco necrosis viruses. The inhibitor is not sedimented after spinning for 2½ hr. at 30,000 r.p.m. Experiments with non-vector insects such as caterpillars have shown that the virus of sugar-beet curly-top, of tobacco ringspot and other viruses, are destroyed within the body of the insect. On the other hand, tobacco mosaic virus passes through the body of the caterpillar unchanged though greatly reduced in concentration. By the use of the specific insect vector and artificial feeding methods it was possible to recover the virus of curly-top 24 hr. after it had been injected into the blood of the caterpillar but the viruses of tobacco mosaic and tobacco necrosis could not be so recovered. Experimental evidence is given to show that the virus of beet curly-top is present in the saliva of viruliferous insects.

ACKNOWLEDGEMENTS. Most of this work was done during the tenure of a Rockefeller Foundation Fellowship, and the writer desires to express his gratitude for the facilities afforded to him at the Rockefeller Institute, Princeton, New Jersey, and at the Division of Sugar Plant Investigations, Riverside, California. Grateful acknowledgements are also due to Dr C. W. Bennett of Riverside for his help and advice with the sugar-beet work, always most willingly given.

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EXPLANATION OF PLATES III AND IV

PLATE III

Leaf of bean (*Phaseolus vulgaris*) inoculated on the right side with tobacco necrosis virus plus inhibitor from earthworm; left side inoculated with the same quantity of virus without inhibitor. The inhibitor used was the supernatant from extract centrifuged for 2½ hr. at 30,000 r.p.m. (Photo. J. A. Carlile.)

PLATE IV

Effect of boiling the inhibitor. Leaf of bean on left is inoculated with tobacco necrosis virus; centre leaf is inoculated with the same quantity of virus to which has been added inhibitor heated to 100° C. for 5 min.; leaf on right inoculated with virus plus unheated inhibitor. (Photo. J. A. Carlile.)

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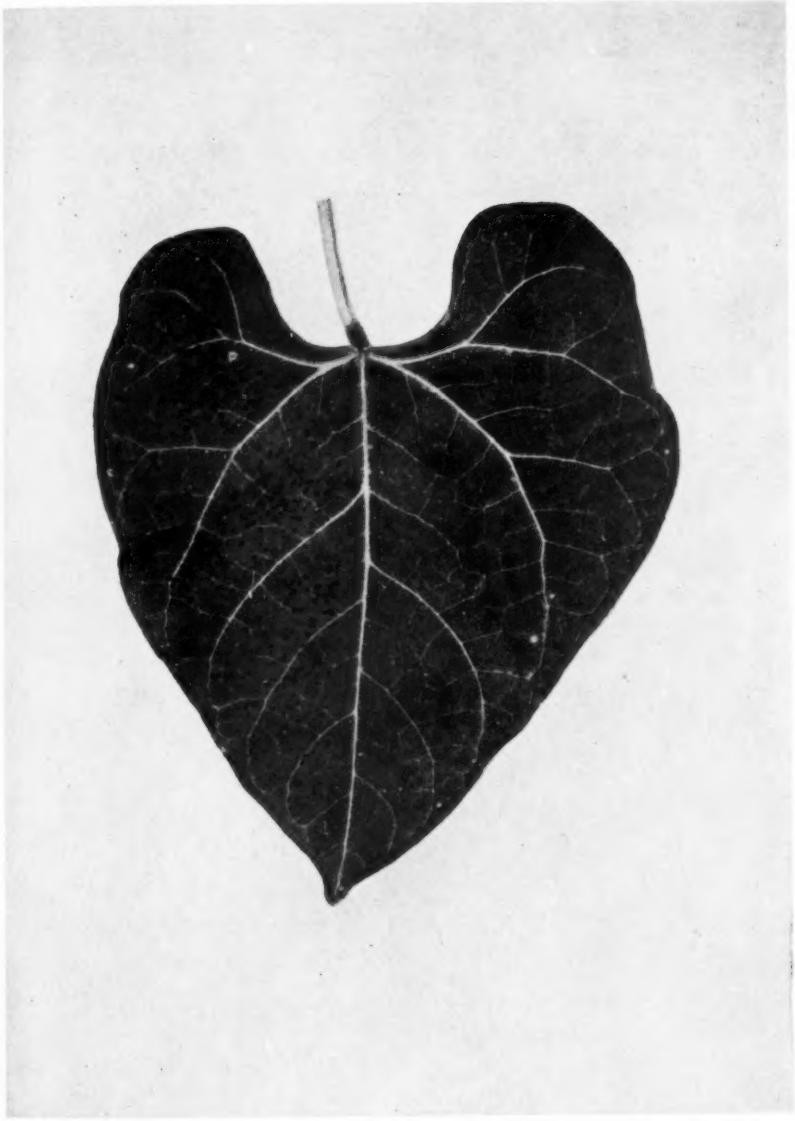

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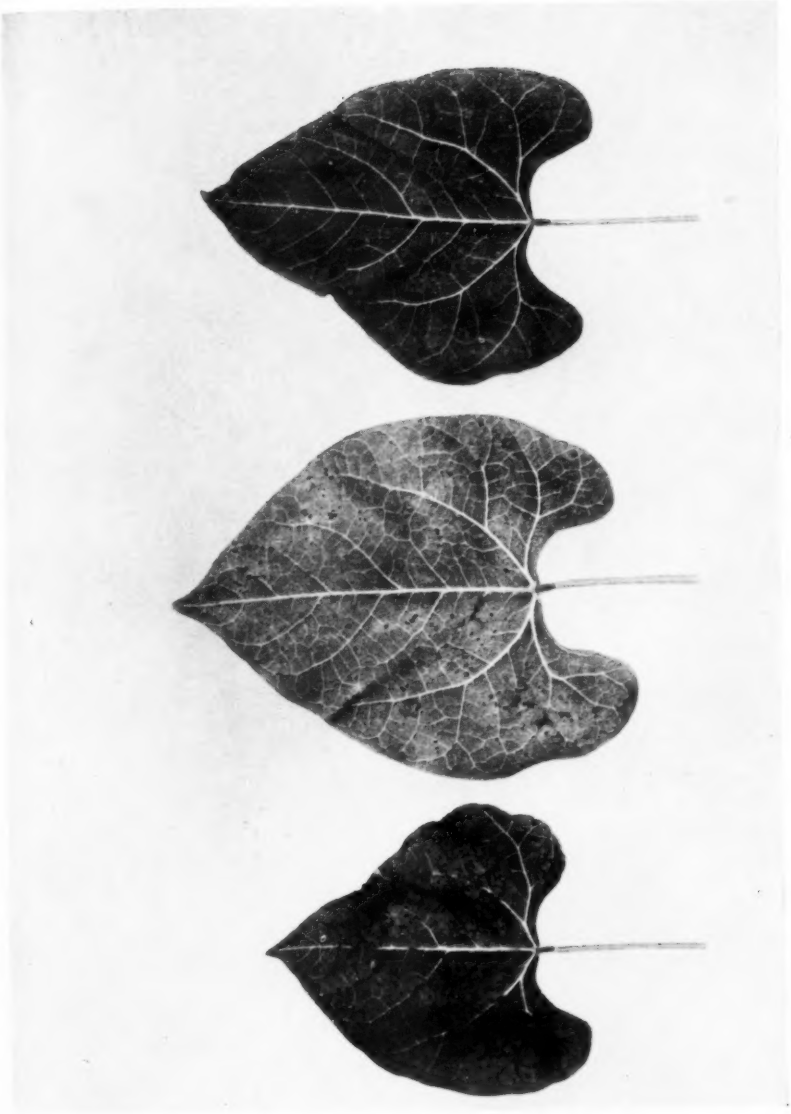

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ON THE OCCURRENCE OF THE CRAB-LOUSE (*PHTHIRUS PUBIS*: ANOPLURA) IN THE HAIR OF THE HEAD

By P. A. BUXTON

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In studying head-lice in large numbers of crops of hair from the scalp (Buxton, 1936, 1938, 1940), a small number of infestations with crab-lice (*Phthirus pubis*) have been discovered. The distribution of crab-lice in over 3000 samples is given in Table 1. It will be seen that *Phthirus* was found in samples of hair from the scalp in every locality studied, except in the small number from Lagos. In the six places in which this insect occurred, the rate of infestation was always below 1%, but always above one in a thousand.

Table 1. *Showing distribution of crab-lice* (Phthirus) *and head-lice* (Pediculus) *in crops of hair from the scalp, from certain localities*

Place	No. of crops examined	*Phthirus* present	%	*Pediculus* %
Lagos, Nigeria	102	0	0	20·6
Sokoto, Nigeria	409	3	0·7	10·3
Kakamega, Kenya	359	2	0·6	25·0
Nairobi, Kenya	415	2	0·5	9·0
Colombo, Ceylon	240	1	0·4	52·1
Jerusalem, Palestine	543	1	0·2	7·2
Cannanore, Malabar	1437	9	0·6	38·0

It would be interesting to know if there is a tendency for those infested with head-lice to be more frequently infested with crab-lice in the scalp. Unfortunately the data for each locality are meagre. Even from Cannanore we have only nine infestations with *Phthirus*: among 543 infested with *Pediculus*, four also had *Phthirus*, and among 894 negative for *Pediculus*, five had *Phthirus*: the test for goodness of fit gives no indication that the two infestations are correlated.

The material available can only teach us a little about the composition of a population of *Phthirus*. In the eighteen samples of scalp hair in which *Phthirus* occurred, there were 112 individuals, giving a mean of 6·2 lice per infestation (range 1–33, only three of the populations exceeding ten). In four cases, no record was made of the number of male, female and larval crab-lice. In the remaining fourteen populations there were thirteen males, thirty-four females and twenty-one larvae. The number of larvae seems very low, and it is possible that our sieves, which certainly retain *Pediculus* in the first instar, may allow *Phthirus* to pass. The total number of populations being so low, no

further analysis of the data seems justified. The only other available information is published by Nuttall (1918): from the pubic hair of one person he recovered 232 adult *Phthirus*, of which eighty-eight (38%) were males. On the whole one may say that, in the composition of a population, *Phthirus* shows a strong resemblance to *Pediculus* (a subject on which a full paper is in preparation).

It is unfortunate that we know nothing of the general abundance of crab-lice in any of the populations from which the samples of scalp hair have been received, neither do we know whether the individuals in whose scalps *Phthirus* occurred were infested in other parts of the body. It would be of great interest if a general survey for head, body and crab-lice could be carried out on a number of individuals.

It is generally known that, though the crab-louse is doubtless commonest in the pubic and perianal regions, it may be found on any hairy part of the body (Nuttall, 1918; Payot, 1920). The facts here set out add a little precision to our knowledge of this insect: they tend also to support the view that, though doubtless often disseminated by sexual contact, this louse has a number of ways of spreading itself through the human community. The view is sometimes held that an infestation with crab-lice, particularly if it occurs in the pubic region, is evidence of sexual activity, and may be evidence of sexual irregularity. From that view I dissent strongly, holding it to be founded on an imperfect knowledge of the insect's biology, and knowing that it has led to unjust conclusions.

SUMMARY

Over 3000 samples of hair from the scalp have been examined from four places in Africa and three in Asia. In every sample (except one small one) infestations of crab-lice (*Phthirus pubis*) were found; in each place they were found in less than 1% of heads.

The view is emphasized that the crab-louse has several ways of spreading through the human community.

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A NEW GENUS AND SPECIES OF MALLOPHAGA

By THERESA CLAY, B.Sc.

From British Museum, Natural History

(With 7 Figures in the Text)

Elsewhere (paper in the Press) the author has shown that the majority of species originally described under *Goniodes*, although somewhat diverse in form, are fundamentally similar and cannot be separated generically. However, there are certain species originally placed in *Goniodes* which are generically distinct and should be separated. Among these is a group of species, forming the new genus described below, which is distinguished in both sexes by the small size of the first segment and the characters of the terminal segments of the abdomen and in the male by the form of the clavi.

Virgula n.g.

Description of the genus. Head circumfasciate and somewhat diverse in shape with the form of the male clavi being the most constant and typical generic character. The clavi (=*zapfen* of Kéler) are transparent and consist of a basal portion which is prolonged distally into a fine point (Fig. 2*a*); in the female the clavi are normal. In the known species the antennae are sexually dimorphic, the male having the distal pre-axial angle of the third segment produced to a greater or less extent. In *Goniodes*, on the other hand, in the sexually dimorphic antennae, it is the distal post-axial angle which is produced.

Pterothorax comparatively large with straight divergent lateral margins and a central sternal plate bearing hairs.

Abdomen elongated and somewhat pointed posteriorly with segment I (=true II) small in both sexes (compare *Goniodes*). In the male segments VIII and IX (=true IX and X) are fused and elongated in an antero-posterior plane. The terminal bilobed portion of the abdomen is probably formed from segment X (=true XI). In *Goniodes* segments VIII and IX are small and not fused and segment X is probably associated with the genital opening which lies on the dorsal surface. In this genus however the genital opening is ventral. Paratergal plates well marked with complicated re-entrant heads. Sternal thickening of segments I–VI in the form of lateral plates; sternites of VIII and IX fused and giving rise to an elongated finger-shaped appendage bearing minute spines especially numerous on the terminal area. At the base of this appendage the chitin is modified to form what is apparently a hinge and there are supporting struts passing in towards the appendage from each lateral margin.

In the female of *Goniodes* segments VIII and IX (=true IX and X) are fused and surround the small remnant of segment X (=true XI). In the genus *Virgula*, it appears that segment X is comparatively large and has well-marked tergal plates separated medianly and that it is not surrounded by but is posterior to the segment formed from the fusion of VIII and IX. The genital region is without particular distinguishing marks and there is a single row of hairs on the posterior margin of the vulva.

Genotype. *Goniodes meleagridis* (Linné) from *Meleagris gallopavo domestica*, the domestic turkey.

This genus contains species from *Meleagris*, *Agriocharis*, *Lerwa*, *Oreophasis*, *Pauxi*, *Ortalis*, *Chamaepetes*, *Crax*, *Penelope*, *Penelopina Dendrortyx*, *Callipepla* and *Odontophorus*. The distribution of this genus is therefore somewhat curious, occurring as it does on the Meleagrididae, on one genus of the Phasianinae (*Lerwa*), on the Odontophorinae and Cracidae. It may also of course be found to occur on other families of birds. This distribution cannot indicate any close affinities between the families and subfamilies mentioned above but may possibly be due to the fact that the genus was once widespread throughout the Galliformes and has since died out in the intervening genera. The diversity of the species of the genus lends support to this theory.

Virgula meleagridis (Linné), 1758 (Figs. 1–4)

Pediculus meleagridis Linné, 1758, p. 613. Host: *Meleagris gallopavo domestica*.
Goniodes stylifer Nitzsch, 1818, p. 294. Host: as above.
Rhopaloceras styliferum Taschenberg, 1882, p. 47, emend. for *stylifer* Nitzsch.

This is a distinct and characteristic species separable from the other species of the genus by the temples in both sexes being greatly prolonged backwards and by the characters of the male genitalia.

Male. Head as shown in Fig. 1*a*. First segment of antennae enlarged and bearing a small thickened process; third segment has the distal pre-axial angle slightly prolonged and the distal dorsal margin bears a small thickened process, giving rise to three small hairs. Temples expanded and produced backwards each side.

Thorax with lateral margins of pro- and pterothorax divergent. Sternal plate triangular in shape and bears six stout elongated hairs each side (Fig. 2*b*). Dorsal chaetotaxy as in female.

Abdomen somewhat elongated with segment I the shortest and with segments VIII and IX enlarged. Tergal plates I–VI are separated widely; those of the terminal segments being transversely continuous. Paratergal plates well developed with large beak-like re-entrant heads. Sternal thickening of segments I–VI in the form of individual lateral plates; that of segments VII and VIII in the form of single central plates. The ventral abdominal appendage arises from segment VIII and bears numerous spine-like hairs on the distal portion (Fig. 1*b*). On the dorsal surface of the abdomen segments

II–VI have one long lateral hair each side; segment I has six central hairs; segments II–VI with number of hairs variable in number, ranging from seven to ten; segment VII has two central hairs. On the ventral surface segment I

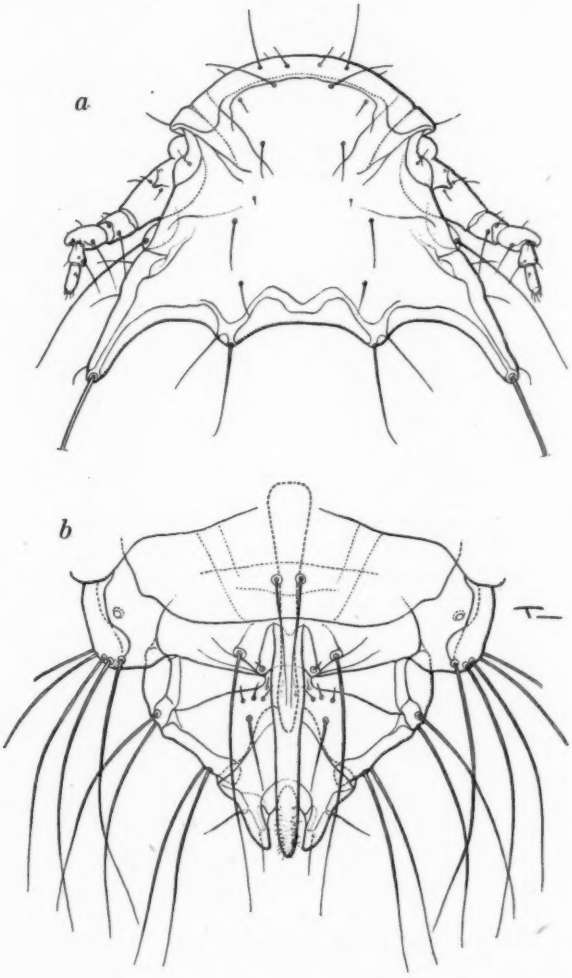

Fig. 1. *Virgula meleagridis*, ♂. *a*, head; *b*, terminal segments of abdomen.

has two central hairs; segments II–V have a variable number of hairs on each segment, ranging from eight to ten; segment VI has four hairs. Terminal segments as shown in Fig. 1*b*.

The genitalia (Fig. 2*c*) have been fully described by Cummings (1916, p. 292).

Female. Head as shown in Fig. 3 and differs from that of male in the absence of the modified clavi and enlarged antennae.

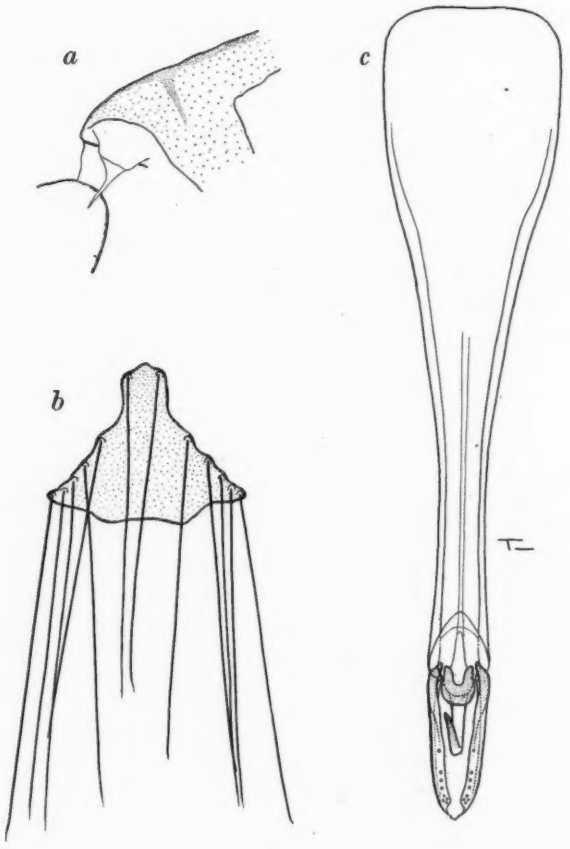

Fig. 2. *Virgula meleagridis*, ♂. *a*, clavi; *b*, sternal thoracic plate; *c*, genitalia.

Thorax and abdomen as shown in figure. On the ventral surface segment I has two central hairs; segment II has fifteen to eighteen hairs across the segment; segments III–V have seventeen to twenty hairs; segments VI–VII have two central hairs. Chaetotaxy of vulva and terminal segments as shown in Fig. 4.

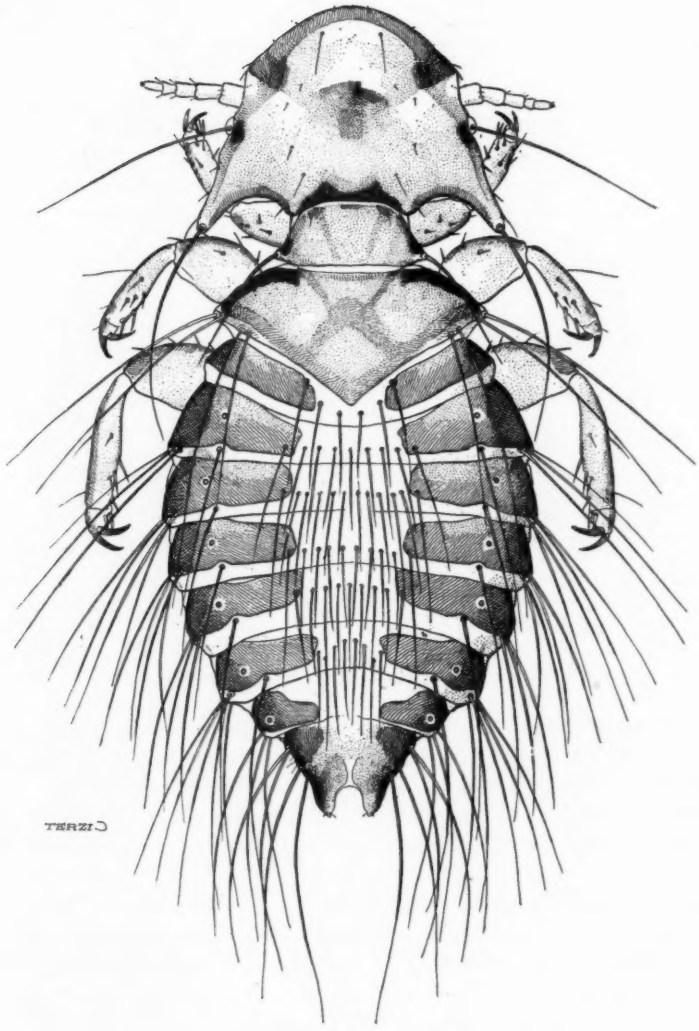

Fig. 3. *Virgula meleagridis*, ♀.

Table 1

	Male		Female	
	Length mm.	Breadth mm.	Length mm.	Breadth mm.
Head	0·76*	1·28	0·81	1·44
Prothorax	0·40	0·66	0·31	0·66
Pterothorax	0·62	1·13	0·68	1·22
Abdomen	2·25	1·50	2·19	1·76
Total	3·85		3·54	
Cephalic index	1·68		1·78	

Total length of genitalia 0·825 mm.

* Measurements taken from anterior margin to mid-line of occiput.

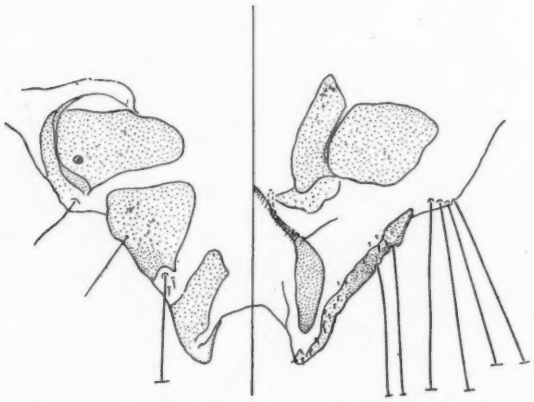

Fig. 4. *Virgula meleagridis*, ♀. Terminal segments of abdomen.

Specimens examined. 10 ♂♂, 8 ♀♀ from *Meleagris gallopavo domestica* from various localities; 9 ♂♂, 5 ♀♀ from skins of *M. gallopavo merriami* Nelson from Texas; 5 ♂♂, 6 ♀♀ from skins of *Agriocharis ocellata* (Cuvier).

Neotype. ♂ in the British Museum Collection, no. 1906–174, from *Meleagris gallopavo domestica* from Roumania. *Neoparatypes.* 9 ♂♂, 8 ♀♀ from the same host from various localities.

Taschenberg (1882, p. 47) included this species in his genus *Rhopaloceras*, for which he made no genotype. Harrison (1916, p. 24) designated *Goniodes aliceps* Nitzsch from *Tinamus tao* as genotype of *Rhopaloceras*, which means that this genus is quite distinct from that described above.

Virgula lervicola n.sp. (Figs. 5–7)

This is a distinct species not closely resembling any other known species of the genus. The diagnostic characters are the shape of the head and terminal segments of the abdomen in both sexes and the male genitalia.

Description of male. Head with narrow clypeal band and transparent pointed clavi characteristic of the genus; antennae with first segment enlarged

Fig. 5. *Virgula lervicola*, ♂. *a*, head and thorax; *b*, paratergite and sternite of fourth abdominal segment.

and bearing small thickened process and with distal pre-axial angle of third segment prolonged slightly with thickened distal end. Temples with angles curved slightly posteriorly and bearing thickened elongated hair (Fig. 5*a*).

Thorax as shown in Fig. 5*a*, with irregular triangular sternal plate bearing four stout elongated hairs each side.

Abdomen somewhat elongated in shape with segment I small and segments VIII and IX enlarged, the latter being deeply bilobed posteriorly and with the distal point of each lobe greatly thickened. Tergal plates I–VI widely separated; plates VII and VIII transversely continuous. Paratergal plates of characteristic form (Fig. 5*b*). Sternal thickening of segments I–VI in the form of individual lateral plates and that of segment VII as a continuous plate

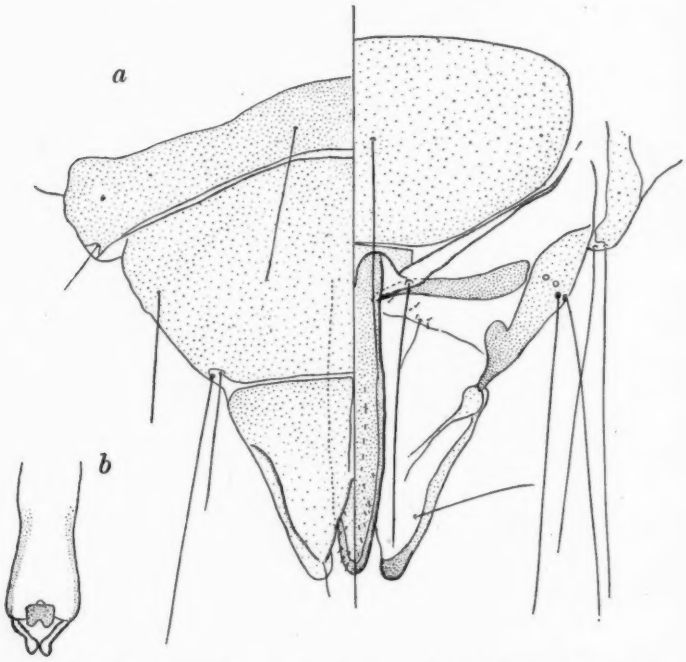

Fig. 6. *Virgula lervicola*, ♂. *a*, terminal segments of abdomen; *b*, genitalia.

across the segment. Ventral abdominal appendage arises from segment VIII and does not reach beyond the posterior margin of abdomen (Fig. 6*a*). Abdominal chaetotaxy as shown in table given below.

Genitalia comparatively small with basal plate swollen proximally and paramera club-shaped (Fig. 6*b*).

Description of female. Head differing in shape from that of male (Fig. 7*a*) and with clavi somewhat transparent and not projecting laterally.

Thorax as in male.

Abdomen with first segment short and terminal segment enlarged, and broadly bilobed. Tergal plates I–VII separated medianly; paratergal plates

as in male; sternal thickening on segments I–VII in the form of lateral plates. Posterior margin of vulva bilobed and set with short hairs (Fig. 7*b*). Abdominal chaetotaxy as shown in table.

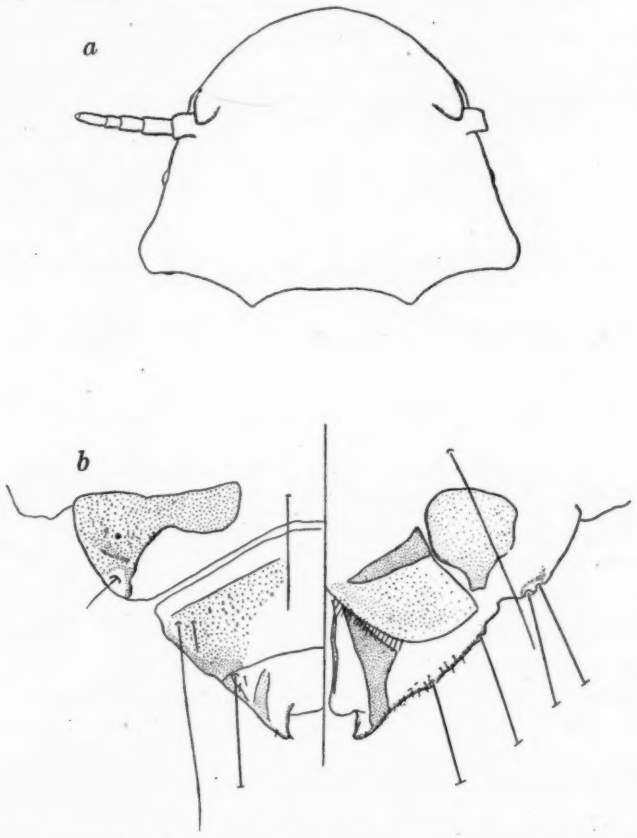

Fig. 7. *Virgula lervicola*, ♀. *a*, outline of head; *b*, terminal segments of abdomen.

Table 2. *Abdominal chaetotaxy*

	Male			Female		
	T.	S.	P.	T.	S.	P.
I	6	2	0, 0	8	2	0, 0
II	1, 6, 1	6–8	1, 1	1, 10–14, 1	10–12	1, 1
III	1, 6, 1	6–8	1, 1	1, 10–14, 1	10–12	1, 1
IV	1, 6, 1	6–8	1, 1	1, 10–14, 1	10–12	1, 1
V	1, 6, 1	6–8	2, 2	1, 10–14, 1	10–12	2, 2
VI	1, 4, 1	2	2, 2	1, 8, 1	2	2, 2
VII	2	2	2, 2	Fig. 7*b*	Fig. 7*b*	2, 2
VIII	4	Fig. 6*a*		Fig. 7*b*	Fig. 7*b*	
IX	4	Fig. 6*a*				

T. = tergal. S. = sternal. P. = paratergal.

Table 3

	Male		Female	
	Length mm.	Breadth mm.	Length mm.	Breadth mm.
Head	0·60–0·65	0·78–0·86	0·69–0·70	0·94–0·97
Prothorax	0·24–0·26	0·48–0·50	0·22–0·24	0·52–0·54
Pterothorax	0·36–0·40	0·72–0·79	0·36–0·38	0·79–0·83
Abdomen	1·42–1·55	0·98–1·09	1·29–1·42	1·11–1·30
Total	2·57–2·87		2·54–2·65	
Cephalic index	1·30–1·32		1·37–1·39	

Total length of genitalia 0·385 mm.

Described from 13 ♂♂ and 18 ♀♀ from skins of *Lerwa lerwa* (Hodgson) from Sikkim.

Holotype. ♂ in the Meinertzhagen collection, slide no. 3119. *Paratypes.* 12 ♂♂ and 18 ♀♀.

The following species should also be included in *Virgula*:

Goniodes longipes Piaget.

Goniodes longipes Piaget, 1880, p. 253, pl. XX, fig. 7. Host: *Pauxi pauxi* (Linné). (*Crax galeata.*)

This species is represented in the Piaget Collection in the British Museum by two males and two females and in the Leiden Museum by two females. It is hoped in a subsequent publication to give figures and a description of this species.

Goniodes bicolor Rudow.

Goniodes bicolor Rudow, 1869, p. 26. Host: *Penelope marail* (Müller). (*Penelope Macalli.*)

Taschenberg (1882, p. 34), who saw Rudow's specimens, considered this species to be identical with *longipes* Piaget. It can therefore be assumed that the two species are congeneric, but without material from the type host of *bicolor* it is not wise to assume that *bicolor* and *longipes* are conspecific.

Goniodes eximius Rudow.

Goniodes eximius Rudow, 1869, p. 25. Host: *Oreophasis derbianus* Gray. (*Oreophasis Derbyanus aus* Guatemala.)

This species was described and figured by Taschenberg (1882, p. 35, pl. III, fig. 1) from Rudow's original specimens and is a typical *Virgula*.

Goniodes diversus Rudow.

Goniodes diversus Rudow, 1870, p. 484. Host: *Penelopina nigra* (Fraser). (*Penelope nigra.*)

Taschenberg (1882, p. 37), who saw a single example of this species from Rudow's collection, considered that it was most probably conspecific with *eximius*. Therefore it can be assumed, as in the case of *bicolor* and *longipes*, that *diversus* and *eximius* are congeneric although not necessarily conspecific.

Goniodes rotundus Rudow.

Goniodes rotundus Rudow, 1869, p. 28. Host: *Penelopina nigra* (Fraser). (*Penelope nigra.*)

It does not appear from the description that this species is conspecific with *diversus*, and it is difficult to say to what genus it does belong. The name must therefore be ignored until sufficient material from *Penelopina nigra* has been examined and the species occurring on this host known.

Species included in the genus Virgula

	Type Host
Virgula meleagridis (Linné).	*Meleagris gallopavo domestica.*
Virgula lervicola n.sp.	*Lerwa lerwa* (Hodgson).
Virgula longipes (Piaget).	*Pauxi pauxi* (Linné).
Virgula bicolor (Rudow).	*Penelope marail* (Müller).
Virgula eximia (Rudow).	*Oreophasis derbianus* Gray.
Virgula diversa (Rudow).	*Penelopina nigra* (Fraser).

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